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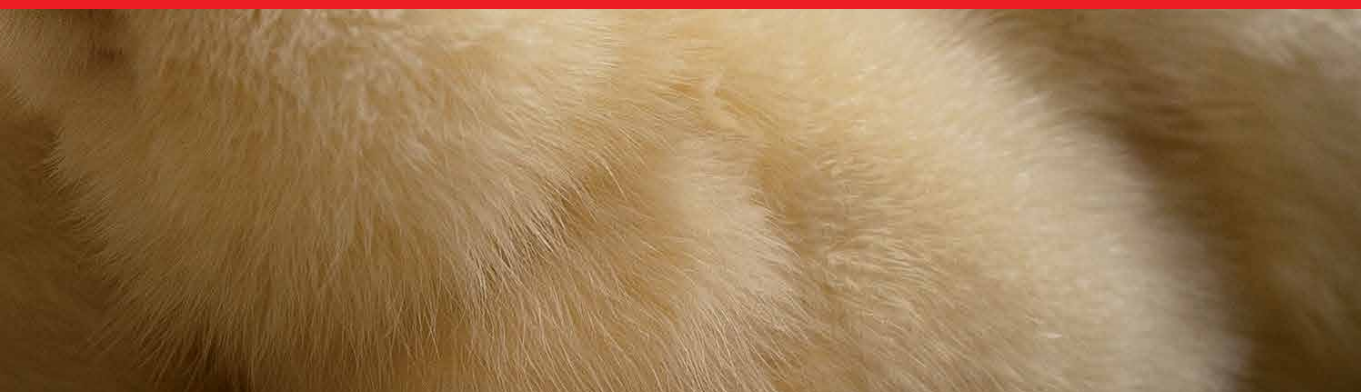
Animal Husbandry

Edited by Sándor Kukovics



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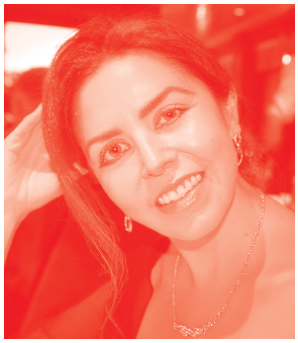

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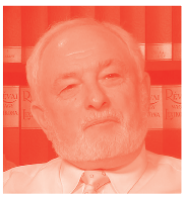
Meet the editor



Prof. Dr. Sándor Kukovics has spent 40 years at the Research Institute for Animal Breeding and Nutrition (Herceghalom, Hungary), where he is responsible for the small ruminants department. He has edited 35 books, published more than 1,100 articles, and obtained four product licenses. In addition to his research work, he is involved in undergraduate and doctoral education at various universities in Hungary. He has been president of the Hungarian Dairy Sheep and Goat Public Utility Association since 1996, and executive manager of the Sheep and Goat Product Board (Hungary) since 2010. Between 2015 and 2019 he served as vice president of the EU COPA-COGECA Working Party on Sheep and Goats, and he has served on the Board of Directors of the International Goat Association since 2016.

Contents

Preface

Animal husbandry is a complex and detailed field covering all aspects of the animal from its housing and feeding, to animal health and welfare conditions, reproductive biology and product production.

Computer programs are of great assistance, enabling the use of available resources and the feasibility and optimization of goals which, thanks to the developments of the last few decades, can now be achieved. Application of these goals is a major part of the production process in developed countries, while in developing countries it is only the case in certain production units.

Many sub-fields affect the keeping of animals and the production process, including nutrition, genetics, reproductive biology, animal welfare and well-being, as well as the production system, the behavioural characteristics of animals, and the production of greenhouse gases. Since the primary purpose of keeping animals is to provide people with adequate food, food of animal origin is closely related to people's health and nutritional status, but also to their employment and livelihoods. As a result, all disciplines related to animal husbandry and breeding are equally important for humans (and their animals).

In the age of genomic selection, the use of traditional breeding methods is still of fundamental importance. Livestock breeding uses favourable parental qualities to continuously improve the production qualities of domesticated animals. The realization of genetic potential is affected by environmental conditions (feeding, animal health conditions, management, etc.). The defining knowledge of this area is summarized in Chapter 1.

To create a properly functioning livestock economy, it is necessary to know the costs of each process as well as the final product yield. The use of software can help to optimize the input materials to achieve the expected quantity and quality of output. Chapter 2 provides an insight into the details and application of such programming.

Animal welfare and animal well-being (wellness) are both important factors in animal husbandry for all animal species. In the case of dairy cows, selection pressure aimed at increasing yield has many adverse animal health consequences. It is important to be aware of the increase in the tendency to diseases that occurs in parallel with the increase in milk yield. Chapter 3 introduces a computer database that enables the development and application of the necessary selection program, based on the knowledge of the genetic information and the animal's wellness characteristics.

The relationship between animal food and human health is presented in great detail in Chapter 4. Livestock farming affects about 600 million smallholdings in developing countries and plays a significant role in the food supply of the people living there. While these farms follow different production systems, their activities also

have significant social and employment implications. Animal products are the primary source of many nutrients (protein, fat, vitamins and minerals, but they also contain fiber) missing from the diet of people who follow a starch-based diet. This phenomenon not only means malnutrition, but also the presence of many diseases in the affected population. The relationship between animal food and human health is presented in great detail in the fourth chapter.

Although the selection is very successful in increasing milk yield in cattle, it has a dramatic negative effect on the fertility of dairy animals. This effect has been evaluated in detail in the case of the Black Holstein-Friesian, the world's most common breed of dairy cattle. The events of the 100 days after calving are decisive for improving the fertility of the herd. During this period diseases and other phenomena appearing in the slaughtered animals are identified, determining the likelihood of the next successful pregnancy. Knowledge of relevant data is particularly important in large dairy herds. The problems of animal fertility, the various test methods that can be used, and their advantages and disadvantages are evaluated in Chapter 5.

In most meat-producing countries, the wide range of animal welfare and treatment requirements, increasingly widely understood in developed countries, remains partially unknown. Stress factors, both on and off the farm, that affect product quality, are analyzed in Chapter 6, including appropriate housing, feeding and health conditions.

Even in the case of farmed animals, the social ranking of individuals is of great importance, not only for access to food (fodder) but also for reproductive opportunities: stronger animals always have an advantage. The defining details of the formation of the social ranking of Zulu rams are presented in Chapter 7.

The horse occupies a special place in the relationship between animals and humans. In addition to working horses, the importance of breeds that can serve both sports and human entertainment has increased in the last century. In addition to the "natural" walk, trot and canter, certain breeds of horses bred for this are also capable of so-called "artificial gaits", such as the fox trot, running walk and rack. Chapter 8 compares horse breeds capable of these special gaits.

In the case of all animal species, understanding the health of the microbiome flora of the digestive system, each part of which has a different microbiome composition, is of fundamental importance for maintaining the appropriate animal feed and animal health status. Chickens are particularly sensitive to any unexpected microbiome changes due to their short life span and high animal density. The details of this process are presented and evaluated in Chapter 9.

In the animal husbandry and production process, compounds may enter animals and their products that pose dangers to both animals and humans. These compounds (polycyclic aromatic hydrocarbons, PAHs) are present in exhaust gases, cigarette smoke, waste, plant protection agents, insecticides, industrial by-products used as feed, etc., and thus also in feed, posing a danger to animal product consumers. Chapter 10 summarizes knowledge of these issues.

Although this book only covers certain issues in animal husbandry, I still trust that readers will be armed with extremely important knowledge that will equip them to understand some aspects of this diverse science.

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Section 1

Breeding

Chapter 1

Basic Animal Breeding Methods

Mohan Singh Thakur

Abstract

In the era of genomic selection, basic animal breeding methods are still playing a very important role in animal selection and their improvement. Animal Breeding involves the selective breeding of domestic animals with the intention to improve desirable and heritable qualities in the next generation. An animal's overall performance is mostly influenced by genetic potential acquired from its parents, as well as the environment, which includes nutrition, health, management, and other factors. This chapter covers a brief outline of traditional breeding methods for the selection of animals and their improvement.

Keywords: criteria, methods, selection, improvement, breeding value, inbreeding

1. Introduction

Selection is one of the important processes for any improvement in farm animals. The breeding merit animal is not often determined by a single character, but more often based on many characters simultaneously. The purpose of selection is to produce elite breeding stocks which act as parents of future generations. The system of selection allows the best animals to act as parents of future generations and culling of undesirable animals from the herd. The animals retained have certain acceptable traits which make them produce more. The breeding of animals is underneath human control, and the breeders decide which individuals shall produce the subsequent generation [1, 2]. The breeding of animals is based upon the fact that certain qualities are genetic; hence valuable qualities are passed on from parents to offspring's. Due to selection, the qualities of animals can be maintained or improved in the next generation [3]. The purpose of selection is to enhance the frequency of desirable alleles and reduce the frequency of unwanted alleles from the herd which in the long run consequences genetic improvement in livestock. The overall performance of an animal is mainly influenced by the genetic potential that is inherited from its parents and the environment which particularly encompass feeding, health, management and so forth.

2. Methodology

Breeding for increased productivity over the past few decades has been very successful in terms of improvement of growth, production and reproduction traits; however, it has also had negative consequences on behavior and welfare [4]. Breeding and genetics are playing an important role in the improvement of domestic animals. Therefore, a broad approach is needed that encompasses both production and welfare traits, even though welfare may not be a primary breeding goal of

the selection scheme. Now, in the era of genomics, breeders have lots of opportunities to collect more precise information on the biological impact of certain breeding decisions. This might help breeders to make more accurate decisions in their selection programs. Genomic tools could also facilitate selection for complex traits, which are frequently not possible to measure on a large number of animals. Looking to this the salient features about selection criteria and methods of selection have been discussed in this chapter.

2.1 Types of selection

Figure 1 is showing the different type criteria and methods of selection that are applied for the selection of animals for a single trait or multiple traits in animal breeding [5, 6].

2.2 Selection for improvement of animals

The manmade selection with certain desirable goal plays important role in the improvement of animal. The different types of artificial selection have been discussed in this chapter along with their merits and demerits. The selection, breeding and propagation of animals by breeders are known as artificial selection. There are two approaches for artificial selection. First is the traditional "breeder's approach" in which the breeder applies "a known amount of selection to a single phenotypic trait" by examining the selected trait and selecting to breed only those that show superior values" of the trait under selection [7]. The second is called "controlled natural selection," which is actually natural selection in a controlled environment [8]. The main purpose of animal breeding is not just to improve individual animals genetically but also to improve the future generation of the animal population [9]. The technique or method used by the breeder to make long-term changes in animals is called selection. Selection is the process in which certain individuals in a population are given an opportunity to produce progeny while others are denied this opportunity [10]. It also decides about how many progenies it should produce and how long they should remain in the breeding population. Selection is an important tool for changing gene frequencies to better-fit individuals for a particular purpose. Selection is not an invention of modern man. It has been going on in nature since life existed in the world. Selection is choosing individuals

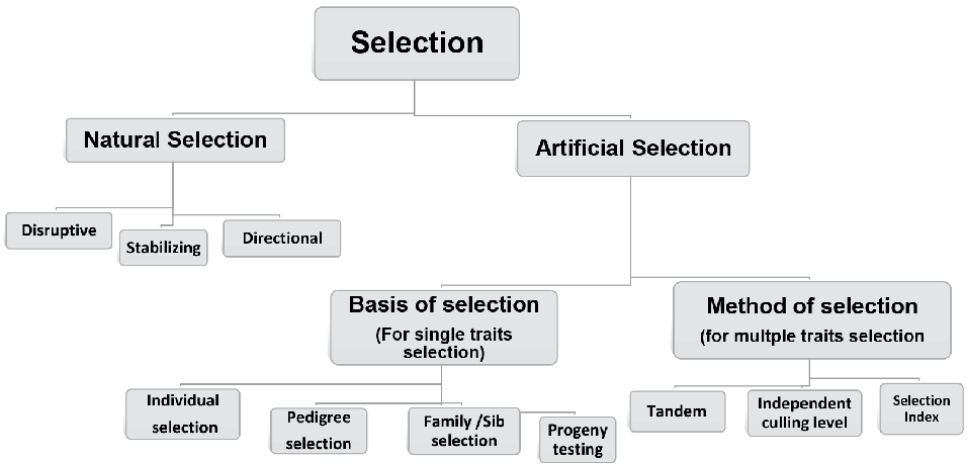

Figure 1.
Different types of selection [5, 6].

that will be parents of the next generation. The effectiveness of selection depends on the ability to recognize those animals, which possess superior inheritance [11]. Those superior animals must be mated together for the production of offspring. The aids available to estimate the breeding value of an animal is through the phenotype of an animal or its relatives.

2.3 Basis of selection for single traits

Figure 2 is showing the different basis of selection that are commonly used to estimate the probable breeding for the selection of animals for a single trait [3, 5, 6].

2.3.1 Individual selection/performance testing

Individual selection is most commonly used as a basis for selective improvement in livestock. Individual selection is based on the performance of individual or individual phenotypic value. These animals are selected based on their own phenotype. Individual selection is more effective when the heritability of traits is high, but the effect decreases with falling heritability. It is the simplest, more rapid and most commonly used basis of selection. If proper performance records are maintained then, the traits like body weight, growth rate, fleece production and other parameters of similar nature can be evaluated directly from the performance of individual animals [10, 12].

2.3.1.1 Selection for qualitative traits

The animals are kept or rejected for breeding purposes based on their phenotype for a particular trait. The progress made in selection depends on how closely genotype is correlated with the phenotype. The phenotype of the individuals is often used to estimate the breeding value for qualitative traits such as color and horned or polled conditions. Selection for such traits based on mass or phenotype is more

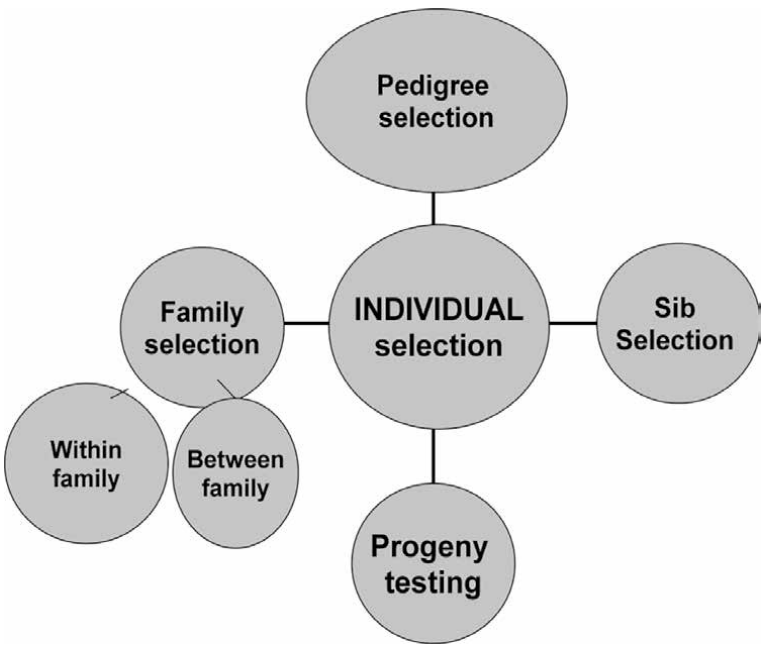

Figure 2.
Different basis of selection [5, 6].

effective than others. For example, in Angus cattle, the coat color Red (rr) is recessive to dominant black (BB) color. But it is practically difficult to distinguish and differentiate the genotype BB and Bb phenotypically. Thus, selection based on individuality will be useful but not always completely accurate [4, 13].

2.3.1.2 Selection for quantitative traits

These traits are controlled by many genes and are also affected by various environmental factors. There is no sharp distinction among the phenotypes and affected by both additive and non-additive gene action. No trait is 100 per cent heritable, because the environment always affects the phenotype to a certain extent. Therefore, the phenotype of an individual for quantitative traits is not the true indicator of genotype. The phenotypic merit of the individuals for quantitative traits is determined by comparing the individual's own phenotype with that of the average of all the individuals within a group from which it is selected and is called trait ratio [14–16].

$$\text{Trait ratio} = \frac{\text{record of individual for a trait}}{\text{Group mean for the same trait}} \times 100$$

The trait ratio depends upon the accuracy of records or available data. The individual's record is of little value unless it shows where the individual ranked relative to others under similar conditions. The environmental part of phenotypic superiority or inferiority will not be transmitted to the offspring or next generation. Therefore, in general, there is a tendency for the average phenotype of the offspring of a phenotypically superior individual will tend to regress toward the average of the population, whereas the average phenotype of the offspring of phenotypically inferior individuals will tend to rise toward the average of the population. Animals own phenotypic value of the character under selection is considered to estimate the probable breeding value (PBV) [5, 13, 14] of that character for that individual.

$$\text{Probable Breeding Value (PBV)} = \overline{P} + h^2 \left(P_i - \overline{P}\right)$$

Where, $\overline{P}$ is population mean, h^2 is heritability, P_i is individual phenotypic value.

Comparison is made with the average of other individuals kept under similar environmental conditions of same age and same time; thus, individuals are ranked relative to others under similar conditions. It is also called as mass selection.

In individual selection, the best animals are selected from within a group of animals of the similar age group that has been reared and treated similarly at the same time, i.e., contemporaries. In individual selection, the breeder will be having a single record of each animal's performance (performance test) and hence an estimate of probable breeding value (PBV) [13] for a given trait is calculated as:

$$\text{Probable Breeding Value (PBV)} = \overline{P} + h^2 \left(P_i - \overline{P_c}\right)$$

Where, $\overline{P}$ is population mean, h^2 is heritability, P_i is individual phenotypic value, $\overline{Pc}$ is average of contemporaries.

2.3.1.3 Advantages of individual selection

- Information of individuals to be selected is easily available [3, 5].
- Used when pedigree information not available, this is the only available guide for selecting the breeding stock.

- Used when generation interval is shorter than progeny testing.
- It gives a direct estimation of BV and is more accurate when h2 is high.
- Traits such as body type, growth rate, fleece production, horn pattern, color and others of a similar nature can be evaluated if suitable records are available.
- Useful for traits expressed in both sexes and performance of the individual is above average for breeding, regardless of the merit of near relatives.
- In the absence of pedigree and progeny records, this is the only available guide for selecting the breeding stock.

2.3.1.4 Disadvantages of individual selection

- Not applied for sex-limited traits such as milk production, egg production, maternal abilities, semen production and litter size, etc. [3, 13].
- Not applied when traits are expressed in later life/after the death of individual.
- Not applied when traits have low heritability, then the individual selection is the poor indicator of breeding value such as reproductive characters.
- Not possible for traits expressed only after sexual maturity, because selection has to be delayed till maturity resulting in waste of time and money.
- The easy appraisal of appearance often tempts the breeder to overemphasis this evaluation in selection.

It is concluded that the individual selection is based on individual's phenotype (appearance) and performance. Individuals are selected solely in accordance with their own phenotypic values. This is the simplest and yields more rapid response. It is the most commonly used method for selective improvement of livestock. Undoubtedly, most of the progress in livestock improvement can be credited to individual selection. Traits such as body type, growth rate, fleece production and other of similar nature can be evaluated directly from the performance of the individual animal, if suitable performance records are being kept; such evaluations are usually available by the time initial selection of breeding stock has to be made. In contrast, only a few can be progeny tested.

2.3.2 Pedigree selection

When the genetic worth or breeding value of animals is determined based on the performance of their ancestors or pedigree information is called as pedigree selection. Pedigree may be a record of an individual's ancestors associated with it through its parents. Therefore, the selection is based on the information of the ancestors of individuals that are related to it. Performance records from ancestors can provide useful information about the potential genetic worth or the breeding value of the individuals in question. This will give useful information before the animal is old. An estimate of calf's potential milk yield could be determined based on the milk yield of its mother until such time as the calf is grown up and can be milked. When adequate information on the merit of the individual is not available, then attention is given on pedigree information for selection of individual. From the

selection point of view, knowledge of the different economic traits of the ancestors is essential [17].

It's usual to expect offspring of outstanding parents to be of superior genetic value than the mean of the individuals of the herd. Each parent transmits only sample halves of its genes to every offspring and just one quarter of genes from each grandparent. So, parents never provide the maximum amount of information about the breeding value of a single individual as individual's performance itself would produce. Unless the performance of the ancestor is well known, selection based on pedigree is meaningless. Distant ancestors of an individual give even less genetic information about the individual's breeding value especially for production traits. The pedigree is often classified into two as direct and collateral [2]. Collateral means those descended from same ancestors.

Selecting a cow based on the performance of its great grandparent is as good as random selection because the relationship is $(0.5)^2$ = 0.125, i.e., only 1/8th of the superiority can be expected in the progenies. It will not do much good to go beyond three generations into pedigree due to the halving process of the chromosomes in each generation [9].

When the pedigree data provides information on the phenotypic and genotypic merit of the ancestors then it is called performance pedigrees. If the selection differential for the ancestor could be presented in the pedigree or if the performance record of the ancestor could be expressed as a percentage of the average contemporaries (Trait ratio), the ancestor's records would be of greater predictive value [9, 10].

Figure 3 is showing the different basis of selection that are commonly used to estimate the probable breeding for the selection of animals for a single trait.

2.3.2.1 Degree of relationship

If ancestors are more closely related to the individual (Parent: ½, Grandparent: ¼ and Great grandparent: ⅛) should receive most emphasis in pedigree assessment [16].

In pedigree selection, the PBV [3] of an individual is estimated on the basis of the performance of his ancestors.

$$PBV = \overline{P_c} + b_{AP}\left(\overline{P}_i - \overline{P_c}\right)$$

Where, $\overline{P}$ is population mean, b_{AP} is regression of additive genetic value or breeding value on phenotypic value, Pi is phenotypic average of individual, $\overline{Pc}$ is average phenotypic value of individual contemporaries,

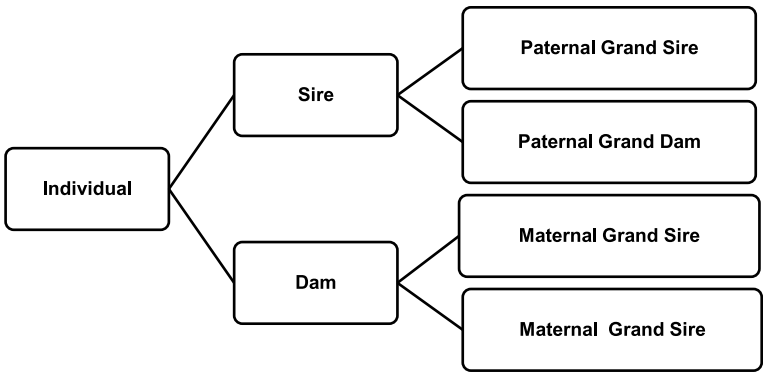

Figure 3.
Schematic of pedigree selection [5].

The selection criteria based on the ancestor's performance is called as the pedigree selection. For pedigree selection, more recent ancestors consider rather than distant.

2.3.2.2 Difficulties in pedigree selection

- Ancestral records are not always available.
- Recording may be faulty due to stray mating
- Most of the characters have low heritability.

The accuracy of pedigree selection when only single information is available for ancestor has been summarized in **Table 1**.

Table 1 summarizes the accuracy of pedigree selection when only single information is available for ancestor (n = 1) [3]. The accuracy of selection based on individuals own record increases, when ancestors' information (parents and grandparents) is combined with an individual's own records.

When information of more than one ancestor are available the accuracy of selection increases, which is described in **Table 2**.

Table 2 summarizes the accuracy of pedigree selection when information of more than one ancestor (n > 1) [3] is available. This increases the accuracy of selection. The pedigree selection is basically only useful to select the individual before its own records is available.

2.3.2.3 Merits

- It is less costly and allows selection at a younger age and provides first-hand information [3, 17].
- It helps in multistage selection and is also useful for sex-limited traits.
- It is useful when two individuals have similar performance.

Ancestor	b	Accuracy of selection (n = 1)
Dam	$0.5\ h^2_D$	$0.5\ h_D$
Sire	$0.5\ h^2_s$	$0.5\ h_s$
Mean of both parents	$1/\sqrt{2}\ h^2$	$1/\sqrt{2}\ h$
One grand parent	$0.25\ h^2$	$0.25\ h$

Table 1.
Accuracy of selection (n > 1) [3].

Ancestor	b	Accuracy of selection (n > 1)
Dam	$0.5\ h^2_D\ [n/1 + (n-1)r]$	$0.5\ \sqrt{}[nh2\ D/1 + (n-1)r]$
Sire	$0.5\ h^2_s\ [n/1 + (n-1)r]$	$0.5\ \sqrt{}\ [nh2\ s/1 + (n-1)r]$
Mean of both parents	$1/\sqrt{2}\ h^2\ [n/1 + (n-1)r]$	$1/\sqrt{2}\ \sqrt{}[nh2/1 + (n-1)r]$
One grand parent	$0.25\ h^2\ [n/1 + (n-1)r]$	$0.25\ \sqrt{}\ [nh2/1 + (n-1)r]$

Table 2.
Accuracy of selection (n = 1) [3].

- The pedigree should be used only as a minor ancestry to individual selection. It may be used to tip the balance between two individuals who are very close on individual merits.

- The selection based on pedigree is only useful than of individual selection only when heritability is moderate or low.

2.3.2.4 Demerits

- All the animals from an inferior pedigree are culled in spite of the fact that an individual may be of good merit and free from recessive alleles [3, 17].

- Some pedigrees get favored irrespective of the true merit of the individuals in the population.

- Pedigree records are from different environmental conditions.

- Pedigree selection provides no basis of selection among the descendants of the same ancestor.

2.3.3 Family selection

Family is a group of individuals that descended from the same ancestor. Family represents a group of animals having common genetic relationship. In family selection, it is presumed that the ancestor has outstanding merit. In animal breeding, generally, the family is a group of animals having a common genetic relationship. In animal population under random mating, generally half sibs (HS) and full sibs (FS) are the most common collateral relatives, whose records are often used to estimate the breeding value. When individual's performance is also included in calculating the sibs average performance, it is called family selection. Family selection is very useful in case of traits with low heritability [1, 3].

2.3.3.1 Sib selection

The selection of individual based on the sibs performance not including individuals own performance.

Based on its sib performance it is of 3 types:

- Full sibs, Maternal half sibs, Parental half sib - cousins, uncle/aunt, nephew/niece.

- Sib selection is performed when the measurements on the individual are not available. For example, Slaughter traits; Sex limited traits; Threshold traits like disease resistance.

HS selection is preferred over FS selection:

- HS are easily available in more number

- The rate of inbreeding can be kept low in HS mating as compared to FS

- FS selection is more likely to be increased by c-effects

Breeding value of sib selection:

$$PBV = \overline{P_c} + rh^2 \frac{n}{1+(n-1)t} \left(\overline{P_s} - \overline{P_c}\right)$$

Where, $\overline{P}$ - Average of contemporaries, $\overline{P_s}$ - average of sibs, n- number of sibs, r- coefficient of relationship (½ for FS and ¼ for HS), t- intra-class correlation (rh^2) among sibs (½ for FS and ¼ for HS), h^2- heritability of the traits, h^2s heritability of sibs = $\frac{nh^2}{1+(n-1)t.}$ [18]

Breeding value of Family selection:

$$PBV = \overline{P_s} + h^2 \left| \frac{1-r}{1-t} \left(P_1 - \overline{P_s}\right) \frac{1+(n-1)r}{1+(n-1)t} \left(\overline{P_s} - \overline{P_{cs}}\right) \right|$$

Heritability of the trait:

- Accuracy of family selection is more for the traits of low heritability [5, 19]
- Accuracy of sib selection based on one FS is 0.5 h and based on one HS is 0.25

2.3.3.2 Advantages of family selection

- Improve the character of low heritability in species with high reproductive rates
- It does not allow generation interval to increase.
- It supports individual selection because it is better to select an individual from a superior family.

2.3.3.3 Disadvantages of family selection

- It is costly
- It requires a large family size depending on the genetic relationship which is only possible in prolific breeder.
- It results in inbreeding and limits the genetic diversity.
- Its accuracy depends upon the genetic relationship among the family members.

2.3.4 Progeny selection

The selection criteria for evaluating an individual based on his progeny performance is known as Progeny selection or progeny testing. Progeny testing is the most important and one of the best criteria of selection. It is regarded as a form of family selection since progenies are the family members of each other. Progeny selection is very useful in the case of sex-limited traits. Such traits are milk yield and fat percentage in cattle and goat, litter size and litter weight at weaning in pigs and egg production in poultry etc. [20]. Progeny selection is also useful for the evaluation of an individual for carcass quality traits which could only be recorded after slaughter. The various functions or equations (sire indices) are used for the estimation of

breeding value of individual. The accuracy of progeny testing is depending on number of progenies tested, heritability of traits and the environmental correlation between the records of different progeny [5].

2.3.4.1 Genetic principle of progeny testing

Each progeny of an individual inherits half of the genes. Hence, the breeding value of the parent is twice the mean deviation of the progenies from population mean.

Points to be considered in Progeny testing:

- Test as many as sires as possible (5 to 10 would be minimal) [5, 13]
- Make sure that dams are mated to sires at random, within age group as possible.
- Produce as many progenies per sire as possible (10 to 15 progenies of either sex for growth traits but up to 300 to 400 progeny is required for traits like calving difficulty and fertility).
- No progeny should be culled until the end of the test.
- Offspring that are being tested are not a select group.
- The performance of an adequate sample of an animal's progeny under normal environmental conditions will give a true indication of its genotype than any knowledge of individuality or pedigree.
- To involve a large number of individuals, Progeny testing should be followed in associated herds.
- Five males should be tested to select one Progeny testing breeding bull
- Ten female progenies of each bull should be performance tested.
- One set of bulls should be completed in two years
- Facility for recording performance of progenies

Constraints in Progeny testing:

- Small Population Size
- Unplanned Mating

Breeding value of Progeny testing

$$\text{PBV} = \overline{\text{P}_\text{c}} + \frac{2\text{nh}^2}{4 + (\text{n} - 1)\text{h}^2}\ (\overline{\text{P}_\text{i}} - \overline{\text{P}_\text{c}})$$

Accuracy of Progeny testing

$$\text{r}_{\text{G}\bar{\text{P}}=}\text{r}_{\text{A}\bar{\text{P}}} = 0.5\sqrt{\frac{\text{nh}^2}{1 + (\text{n} - 1)\text{t}}}$$

Restriction on Records		Heritability	
		Low (h^2 < 0.20)	**High (h^2 > 0.40)**
For selection of Males:			
1	None	Progeny	Own
2.	Females only (i.e., MY)	Progeny	Progeny, maternal relative
3	Relative only (i.e., carcass traits)	Progeny	Sibs, progeny
For selection of Females:			
1	None	Own, Pedigree	Own
2.	Males only (i.e., Semen Production)	Sibs, Pedigree	Sibs, Pedigree
3	Relative only (i.e., carcass traits)	Sibs, Pedigree	Sibs

Table 3.
Choice of records for the optimum breeding program [4, 9].

Where, r is Coefficient of relationship between sire and his progeny, n is Number of progenies, P_i is Mean performance of progenies of i^{th} sire, P_c is Mean performance of contemporaries of progenies of i^{th} sire, h^2 is Heritability of the trait

The choice of records for the optimum breeding program for low and high heritable traits has been summarized in [5, 14, 21] (**Table 3**).

Table 3 [4, 9] summarizes the appropriate criteria for selection male and female for optimum breeding program under different restrictions of records for low and high heritable traits.

Advantages of Progeny testing:

- It is the better method for sex-limited traits, the traits with low heritability and slaughter traits.
- The bulls carrier of recessive gene can be identified by mating with its progenies.
- It evaluates carcass traits that demands sacrifice of animal.
- Progeny testing increases selection intensity.
- Its accuracy increases with an increase in the number of progenies.

Limitations/disadvantages of progeny testing:

- High cost and time are required.
- It increases the generation interval and due to longer generation interval genetic gain per year is low.
- It requires an adequate number of progenies to be tested on a bull.

3. Systems of breeding

Systems of breeding can be classified into two major groups: Inbreeding and Out breeding [5, 22].

3.1 Inbreeding

Inbreeding is the mating of animals more closely related to each other than the average relationship with in the population concerned. The mated individual should have one or more common ancestors in their pedigree up to 4–6 generations. Inbreeding includes mating like parent-offspring, brother–sister. Inbreeding is classified into two types: close Inbreeding and line breeding [22].

Close inbreeding is mating between sibs or between parents and progeny to achieve inbred lines with a relatively high degree of homogeneity. Most of the time, we use the full sib mating method. The same effect can be achieved by consistently back crossing the progeny to the younger parents. Line breeding is a milder form of close inbreeding is in which the relationships of mated individual is kept as close as possible to some ancestor. As a general rule sire is not mated to its daughters but half sib mating is made among the offspring of the particular sire. Line breeding was used extensively in the past in development of British breeds of cattle such as Angus, Hereford and Shorthorn [3]. Line breeding should be practiced in purebred populations of the high degree of excellence, after identifying outstanding individuals and it can be advocated to form a new breeds.

Inbreeding is that it makes more pairs of genes in the population homozygous irrespective of the type of gene action involved. Inbreeding does not increase the number of recessive alleles in a population but merely brings to light through increased homozygosity. When the animals are homozygous for several traits, the regularity of inheritance is assured (i.e., it fixes the characteristics). Inbreeding reduces vigor or it results an inbreeding depression.

Despite certain obvious disadvantages of inbreeding, there are certain instances where it may be used as the advantage of livestock production. It is used to maintain genetic purity and thereby to increase prepotency. It is also used to develop inbred lines and also to eliminate undesirable recessives from the population. When a sire is mated to at least twenty of its daughters and does not produce any recessive characters in the offspring, it may conclude that the sire is not heterozygous for recessive characters.

Inbreeding is to be practised only when the herd is better than the average, i.e., when the frequency of desirable genes is more, herd has an outstanding sire, the breeder knows the merits and demerits of inbreeding and the herd is not maintained for commercial purpose.

3.2 Out breeding

Out breeding is the mating of animals that are less closely related to each other than the average of the population. Its general, effects are the opposite of those of inbreeding. Out breeding increases the heterozygosity of the individual. The maximum practical usefulness of out breeding systems is the production of animals for market. Out breeding is a form breeding where the mates are chosen based on not being related. The following type of out breeding is used in animal breeding [3, 6].

3.2.1 Selective breeding

The selective breeding is used to maintain the purity of the breed along with their improvement. **Figure 4** describes the brief information for genetic improvement of indigenous cattle breeds by selective breeding is shown below:

Figure 3 is showing the different basis of selection which are commonly used to estimate the probable breeding for selection of animals for single trait.

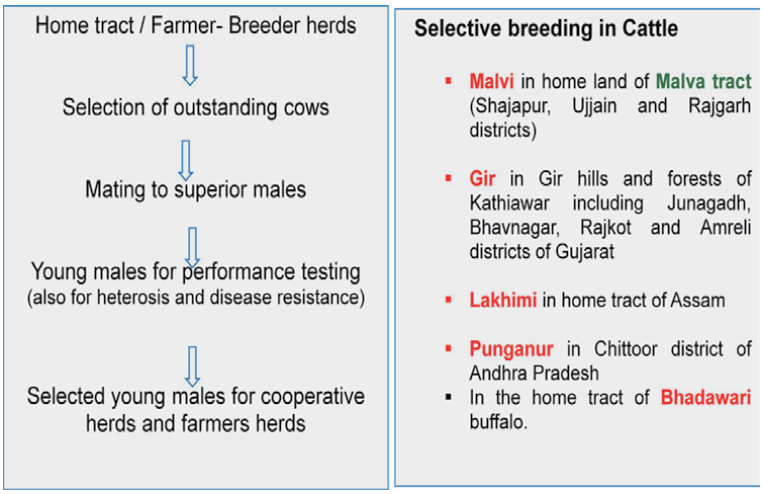

Figure 4.
Schematic of selective breeding [3, 5].

3.2.2 Out crossing

It is usually applies only to mating within a pure well-defined breed. If two lines within the same breed are separated for 4 or 5 generations and the sire from one herd is used in another herd that accounts to out crossing. It is used when the genetic variability and there is lack of selection response [3]. It introduces new genes in the population with reference - color, horn type, etc.

3.2.3 Top crossing

It refers to the use of highly inbred sires to the dams of the base population or non-inbred population within the same breed. It usually refers to the best sire in a pedigree. It also refers to the continued use of sires to different families within a pure bred, same breed or different breed [3, 5].

3.2.4 Up-grading

Grading up or upgrading is the repeated use of pure breed sire (or sires) over females of non-descript population. There is a noticed improvement in crosses if sires from a particular breed (A) are repeatedly back crossed to another breed/non-descript animals (B). Five generations are sufficient to raise the level of inheritance of breed A to 96.9% (0.969) in the fifth generation. After five generations of repeated back crossing to a particular breed, the animals after the end of fifth generation become eligible to be registered as purebred. After 7 to 8 generations of continuous grading up the non descript population will be transferred into well defined purebreed [3, 4].

The level of inheritance (%) of pure-bred male and non-descript in different generation under upgradation program is summarizes below in the [4, 5] (**Table 4**).

Table 4 [4, 5] summarizes the change in the per cent level of inheritance of pure-bred male and non-descript female in different generation under upgradation program. By the successive backcrossing from one population into another population over generation after generation (7–8 generations), the non-descript population can be substituted by pure bred population.

The representative model for upgrading the local cattle by frozen semen and nucleus breeding unit is summarized in [4, 5] (**Figure 5**).

Generation	Level of inheritance (%)	
	Pure breed male (A)	Non-descript females (B)
First generation	50.00	50.00
Second generation	75.00	25.00
Third generation	87.50	12.50
Fourth generation	93.75	6.25
Fifth generation	96.87	3.13
Sixth generation	98.44	1.56
Seventh generation	99.24	0.76
Eighth generation	99.62	0.38

Table 4.
Up-gradation of non-descript population [4, 5].

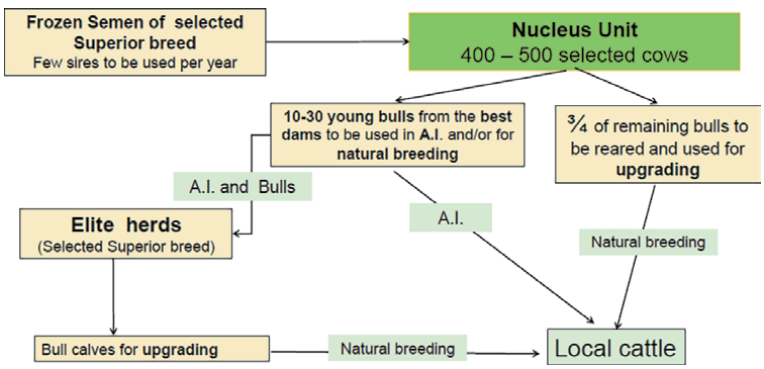

Figure 5.
Representative model for upgrading the local cattle by frozen semen and nucleus breeding unit [5].

3.2.5 Cross breeding

Cross breeding is mating of two individuals from different distinct breeds. In recent years, crossbreeding has been used for development of new breeds or synthetics strain. For example: Santa Gertrudis, Jamaica Hope, Norwegian Red and White, Australian Milking Zebu, Hissardale, Karan Swiss, Sunandini, Taylor breed

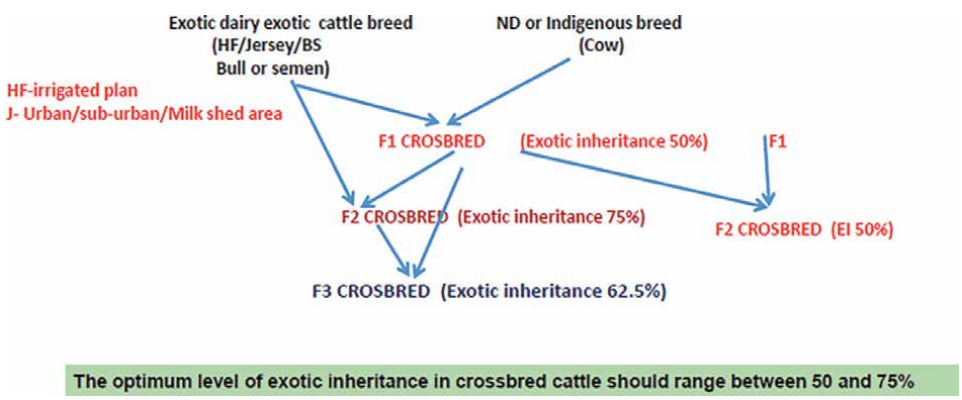

Figure 6.
Representative cross for genetic improvement of non-descript zebu cattle by crossbreeding unit [5].

etc. [4, 5]. The representative diagram for genetic improvement of non-descript zebu cattle by crossbreeding is shown in **Figure 6**.

Crosses of animals from different breeds result in offspring whose level of production is above that of the average of the parents. The increased production may be due to increased fertility, increased pre and post-natal viability, faster and more efficient growth, improved mothering ability, etc. The increased level of performance as compared to the average of the parents is known as heterosis or hybrid vigor. Heterosis is due to non-additive gene action. The breeds or lines with good nicking ability or combining ability are crossed to exploit heterosis.

4. Conclusions

In any species of livestock, the primary aim of breeder is to improve the production traits. In these traditional methods of selection along with intervention of recent molecular techniques has paved the way to exploit the genetic potential of animals upto certain limit. However, in this rapid race of genomics ethical and environmental issues should be taken into consideration.

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Chapter 2

Design and Development of Self-Made Cost-Effective Microsoft Excel Visual Basic Application for Livestock Ration Formulation

Vishal Patil, Ravinder Singh Kuntal, Duraisamy Rajendran and Radha Gupta

Abstract

One of the most important aspects of the livestock sector is ration cost optimization, which results in profit and ideal animal health. Manually preparing rations is time consuming and unsafe. Whereas computers can quickly formulate a ration that meets all of the nutritional requirements, after giving standard data on feeds. However, the existence of the ideal computer programme is questionable; if it exists, it is more expensive, less user-friendly, exclude local feeds, be limited to a particular region/country, feed composition may differ. As a result, in this chapter, the user will learn how to create and develop a self-made least-cost ration formulation using the locally available feeds, so that user may easily build their computer Programme using Visual Basic application of Microsoft Excel. There are three phases to ration formulation for any animal (ruminant or non-ruminant). The first phase requires the user to know the available feeds and their nutrient composition. The second part involves determining which nutrients are important for animals and creating nutrient equations. The third phase involves the creation of a linear programming model. Finally, the interface is being designed. Each phase is thoroughly explained in excel, with suitable data and reference coding.

Keywords: least cost ration, linear model, nonlinear model, visual basic application, nutrient requirements

1. Introduction

The primary goal of ration formulation in animal production is to offer a balanced diet that supports physiological functions such as growth, maintenance, reproduction, and lactation while also providing energy for physical and metabolic activity [1]. A standard and efficient feed formulation must include all the classes of feedstuffs (Animal Nutrition Group, India) [2] as provided in **Figure 1**.

A concentrate is a feed or feed mixture that provides increased levels of primary nutrients (protein, carbohydrates, and fat) while containing less than 18 percent

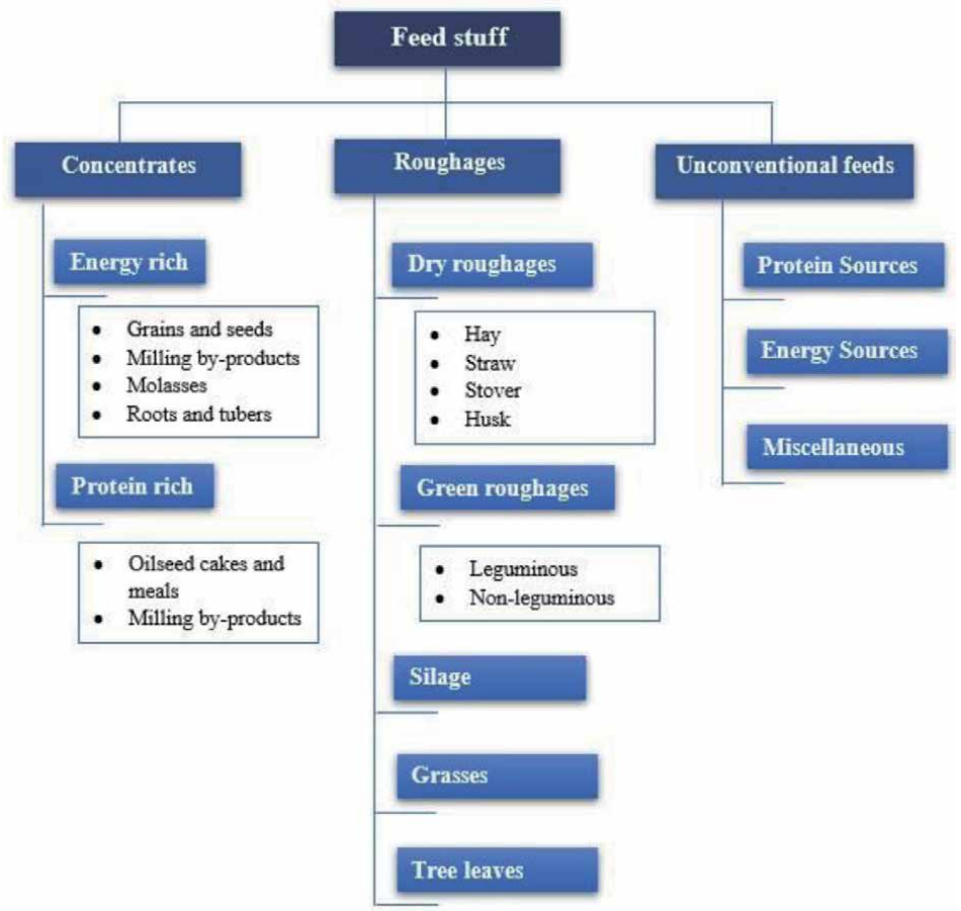

Figure 1.
Different classes of standard feedstuff must be included in the feed formulation according to animal nutrition group, India.

crude fiber (CF) and low moisture. These are high in nitrogen-free extract (NFE) and Total Digestible Nutrients (TDN) and low in crude fiber [3]. There are classified into two categories: energy-rich concentrates and protein-rich concentrates, based on the content of crude protein (CP). When the crude protein content of dry concentrates is less than 18 percent, they are categorized as energy-rich concentrates, and when the CP value is greater than 18 percent, they are defined as protein-rich concentrates [4, 5].

Roughages are heavy foods that contain substantially less digestible material, such as crude fiber greater than 18% and a low TDN content (about 60%) on a dry basis. Roughages differ in the level of protein, mineral, and vitamin composition. Some roughages, particularly legumes, are good suppliers of calcium and magnesium [6]. The phosphorus concentration is likely to be moderate to low, whereas the potassium content is likely to be high, these concentrations are affected by the plant species, soil, and fertilization strategies all have an impact on trace minerals. Roughages are categorized into two classes based on moisture content: dry and green or succulent roughages [7]. Green roughages often have 60–90 percent moisture, while dry roughages have just 10–15 percent moisture. Green roughages are divided into several types for ease of use, including pasture, produced fodder crops, tree leaves, and roots. Based on the nutritional content and preparation methods, dry roughages have been further categorized as hay and straw [8].

Minerals available in the feeds are of different types i.e., Micro minerals, macro minerals and chelated minerals. Microminerals, also known as trace minerals, are needed in milligrams (mg) or microgram (g) quantities [9]. They're found in trace amounts in animal tissues and feeds. They're frequently found in enzyme cofactors and hormones. Cobalt, iodine, zinc, copper, manganese, and selenium are examples of micro or trace minerals. Macro-minerals play a specific role in the formation and function of the animal's body. Animals require the following seven macro-minerals: calcium (Ca), phosphorus (P), sodium (Na), magnesium (Mg), potassium (K), sulfur (S), chlorine (Cl) [10]. Chelated minerals are a class of organic minerals that are divided into proteinates, chelates, and other complexes based on their molecular structure [11]. A chelated mineral is one that has two or more chemical interactions with peptides or amino acids, such as copper or zinc. Each one has a different level of absorption and effectiveness.

The National Research Council has studied nutrient requirements based on several criteria such as dry matter intake (DMI), total digestible nutrients (TDN), crude protein (CP), metabolic energy (ME), calcium (Ca), phosphorus (P), and other elements that affect intake and techniques of prediction [12]. The entire weight of feed minus the weight of water in the feed is expressed as a percentage and is known as dry matter. In feeding studies, dry matter intake is determined by weighing the whole ration supplied and the amount of feed left by the animal [13]. The term "total digestible nutrients" stems from an old system of calculating available energy in feeds and animal energy requirements using a complex calculation of measured nutrients. The whole amount of protein present, determined from the total nitrogen available, is referred to as a crude protein. The percent nitrogen is multiplied by 6.25 to get the percent protein. The digestible energy intake minus the energy in the urine minus the energy in the gaseous result of digestion equals the metabolizable energy. Calcium is required for bone formation, neuron function, and the production of milk and eggs in animals. Phosphorus is also included in a wide range of co-enzymes, nucleic acids, proteins and amino acids [14].

It is essential to know the significance of these nutrients in animal feed. Animal disease control, as well as feed and fodder shortages, are the most pressing issues in animal husbandry. Farmers frequently encounter the following issues.

1. Nutrient requirement for the livestock animal,

2. The amount of feed that must be supplied every day to boost productivity,

3. Feed ingredient costs must be kept under control.

Animal nutrition is necessary for livestock production to be effective. Animal feed efficiency and growth rate can both benefit from good nutrition. Diets that meet the demands of animals must be provided.

This work used goats to fully understand nutritional needs and feed composition through the use of a visual basic application. The three phases of the study are explained in this chapter, the first phase is the selection of feeds and understanding their nutrient composition. The second part involves determining which nutrients are important for animals and creating nutrient equations. The third phase entails the establishment of a linear programming model, followed by the design of the interface.

2. Material and methods

2.1 Data collection

Goats can grow well and produce maximum milk if balanced and nutritious food is fed [15]. A balanced ration should contain digestible nutrients, vitamins, and

minerals, including concentrate feeds and green and dry roughages. The feed list was created based on the most commonly used feeds, fodders and its nutrient composition was used as per ICAR (2013) given in **Table 1**.

2.2 Nutrient requirements and estimation of nutrient requirements

Nutrient requirements are estimated based on the Indian Council of Agricultural Research (ICAR) (2013) and it was programmed in Excel VBA. A balanced ration should meet the nutrient requirement. If the growing goat does not get the nutrients, it will affect milk yield and weight at the time of slaughter [16, 17]. The nutrient requirements for the growing goats are given in **Table 2**. There are three major factors of balanced ration: DMI, CP and TDN.

Dry Matter Intake (DMI) (kg/d): Dry matter intake is dependent upon many factors like fodder quality and quantity, climate condition, and nutrient requirement of goat. The DM requirements of goats for different body weights and growth rate functions are different [18]. The dry matter requirement is calculated based on body

	sl. No	Name of the ingredients	Cost*	DM %	CP %	TDN %
Roughages	1	Bajra -Napier Green grass	0.5	20	8	52
	2	Maize Green fodder	0.5	20	8	60
	3	Multicut sorghum green fodder	1	90	7	50
	4	Lucerne Green Fodder	0.7	20	15.8	60
	5	Wheat straw	1	90	3.3	42
	6	Maize stover	0.9	90	3	42
	7	Ragi Straw (Dry)	0.9	90	6	42
	8	Cereal straw	1	90	3.5	40
Concentrate	10	Maize	1.7	90	8.1	79.2
	11	Wheat	1.8	90	8	75
	12	Wheat Bran	2.2	75	12	70
	13	Soya DOC	3	90	42	70
	14	cotton seed	2.2	90	16	72.2
	15	Cotton DOC	1	90	18	45
	16	Copra DOC	3	90	22	70
	17	Concentrate Mix Type I	3	90	22	75
	18	Concentrate mix Type II	2.5	90	16	70
Minerals	19	Urea	1	95	287.5	0
	20	MM (Mineral Mixture)	6	90	0	0
	21	DCP	3.8	90	0	0
	22	Trace Mineral Mixture	3.5	98	0	0
	23	Salt	0.5	90	0	0

**Cost of the ingredients can be changed in the application.*

Table 1.
Composition of most commonly used feeds, fodders and its nutrient composition (DM, CP, TDN) was used as per ICAR (2013) and present cost of the ingredients.*

Body weight (BW) (kg)	Body weight gain (g/d)	Daily Dry Matter Intake (DMI)		Energy Requirements		Protein Requirements
		Kg	% BW	TDN kg/d	ME	CP
					Mcal/d	g/d
5	0	—	—	0.101	0.36	19
5	25	0.2	4	0.141	0.51	31
5	50	0.21	4.2	0.181	0.66	42
5	75	—	—	0.221	0.8	53
5	100	—	—	0.262	0.95	65
10	0	—	—	0.169	0.61	33
10	25	0.36	3.6	0.21	0.76	44
10	50	0.37	3.7	0.25	0.9	55
10	75	0.35	3.5	0.29	1.05	67
10	100	—	—	0.33	1.19	78
10	125	—	—	0.371	1.34	89
10	150	—	—	0.411	1.48	100
15	0	—	—	0.229	0.83	44
15	25	0.45	3	0.27	0.98	56
15	50	0.5	3.3	0.31	1.12	67
15	75	0.5	3.3	0.35	1.26	78
15	100	—	—	0.39	1.41	89
15	125	—	—	0.431	1.56	101
15	150	—	—	0.471	1.7	112
20	0	—	—	0.285	1.03	55
20	25	0.58	2.9	0.325	1.17	66
20	50	0.6	3	0.365	1.32	78
20	75	0.62	3.1	0.405	1.47	89
20	100	0.62	3.1	0.446	1.61	100
20	125	—	—	0.486	1.76	111
20	150	—	—	0.526	1.9	123
25	0	—	—	0.337	1.22	65
25	25	0.68	2.7	0.377	1.36	76
25	50	0.71	2.8	0.417	1.51	88
25	75	0.73	2.9	0.457	1.65	99
25	100	0.74	3	0.498	1.8	110
25	125	0.71	2.8	0.538	1.94	121
25	150	—	—	0.578	2.09	133
30	0	—	—	0.386	1.39	75
30	25	0.77	2.6	0.426	1.54	86
30	50	0.8	2.7	0.466	1.69	97
30	75	0.83	2.8	0.507	1.83	108

Body weight (BW) (kg)	Body weight gain (g/d)	Daily Dry Matter Intake (DMI)		Energy Requirements		Protein Requirements
		Kg	% BW	TDN kg/d	ME	CP
					Mcal/d	g/d
30	100	0.84	2.8	0.547	1.98	120
30	125	—	—	0.587	2.12	131
30	150	—	—	0.627	2.27	142

Table 2.
Dry matter intake, energy and protein requirements of Indian goat based on body weight (kg) and body weight gain (g).

weight and average daily gain as per the Indian standard [19]. The total DMI intake calculated is in terms of 'kg' and the formula used in VBA code is given below:

$$Dim\ a\ As\ Integer$$
$$Dim\ z\ As\ Integer$$
$$Dim\ y\ As\ Integer$$
$$z = TxtADG.Text$$
$$a = TxtWeight.Text$$

$If\ a < 10\ Then$
$TxtDMIKg.Text = ((a \times 4.1)/100)$
$ElseIf\ a < 15\ Then$
$TxtDMIKg.Text = ((a \times 3.6)/100)$
$ElseIf\ a < 20\ Then$
$TxtDMIKg.Text = ((a \times 3.3)/100)$
$ElseIf\ a < 25\ Then$
$TxtDMIKg.Text = ((a \times 3.025)/100)$
$ElseIf\ a < 30\ Then$
$TxtDMIKg.Text = ((a \times 2.84)/100)$
$ElseIf\ a < 35\ Then$
$TxtDMIKg.Text = ((a \times 2.8)/100)$
$Else$
$TxtDMIKg.Text = 1$
$End\ If$

Energy: Energy is expressed as "Total Digestible Nutrients" (TDN). Energy allows doing physical reproductivity. It also provides for the development, lactation, reproduction, and other physiological functions such as feed digestion [20]. The TDN is calculated based on metabolic body weight and average daily gain as per the Indian standard (ICAR 2013).

From the **Table 1**. Will find CF1 (common factor 1) for 5, 10, 15, 20, 25, 30 kg with respective metabolic body weight (BW^0.75). the formula of CF1 is given below in eq (1)

$$CF1 = \frac{TDN(kg/d) \times 1000}{BW^{0.75}} \quad (1)$$

CF1 values for 5, 10, 15, 20, 25, 30 kg are 30.21, 30.05, 30.04, 30.14, 30.14, 30.11 respectively. As we can observe CF1 values for 5 kg is 30.21 and values for 10 and

15 kg are taken as 30.04 (average), for more than 15 kg can be taken as 30.13 (average of 3).

Then we find CF2 (common factor 2) with respective average daily gain (ADG) by the following formula (eq (2))

$$CF2 = \frac{TDN(kg/d) \times 1000 - \left(BW^{0.75}\right) \times CF1\ values}{BW^{0.75}} \tag{2}$$

CF2 values are found to be 1.6. Therefore (see eq (3))

$$TDN\left(\frac{g}{d}\right) = \left(BW^{0.75}\right) \times CF1 + ADG \times CF2 \tag{3}$$

ME (Mcal/d) can be found by using the following formula (eq (4))

$$ME(Mcal/d) = \frac{TDN(g/d)}{1000 * 0.28} \tag{4}$$

Where 0.28 is the common factor.

If $a < 10$ *Then*
$TxtTdn.Text = (a^{0.75}) \times 30.21 + z \times 1.6$
ElseIf $a < 20$ *Then*
$TxtTdn.Text = (a^{0.75}) \times 30.04 + z \times 1.6$
Else
$TxtTdn.Text = (a^{0.75}) \times 30.13 + z \times 1.6$
End If
$y = TxtTdn.Text$
$TxtMe.Text = (y/1000)/0.28$

Protein: Protein is expressed as crude protein (CP). It is one of the major nutrients in terms of nutrition and cost. CP represents the percentage of protein present in feedstuff. CP is essential for maintenance, increasing the forage intake [21, 22]. It varies for every stage of goat life. Therefore, setting CP level is very important. If the balanced ration does not meet the CP requirement, protein supplies are to be used at a greater cost [23]. At the same time, they were producing a balanced ration for these four requirements to be met compulsorily with the least cost [24]. Then the production will be more and farmers will be benefitted. The CP is calculated based on metabolic body weight and average daily gain as per the Indian standard (ICAR 2013).

From the **Table 1**. Will find CF1 (common factor 1) for 5, 10, 15, 20, 25, 30 kg with respective metabolic body weight (BW^0.75). the formula of CF1 is given in eq (5).

$$CF1 = \frac{CP(g/d)}{BW^{0.75}} \tag{5}$$

CF1 values for 5, 10, 15, 20, 25, 30 kg are 5.68, 5.87, 5.77, 5.82, 5.81, 5.85 respectively. As we can observe CF1 values for 5 kg is 5.68 and values for 10 kg–30 kg are taken as 5.82 (average),

Then we find CF2 (common factor 2) with respective average daily gain (ADG) by the following formula (eq (6))

$$CF2 = \frac{CP(g/d) - \left(BW^{0.75}\right) \times CF1\ values}{BW^{0.75}} \tag{6}$$

CF2 values are 0.46 for less than 10 kg and 0.44 for greater than 10 kg. Therefore (see eq (7))

$$CP(g/d) = \left(BW^{0.75}\right) \times CF1 + ADG \times CF2 \tag{7}$$

If $a < 10$ *Then*
$TxtCp.Text = (a^{0.75}) \times 5.68 + z \times 0.46$
Else
$TxtCp.Text = (a^{0.75}) \times 5.82 + z \times 0.44$
End If

2.3 Research design

The developed tool (RBT) uses VBA (Visual Basic Application) as front end and back end as excel. It is a simple excel file that is saved as .xlsm form and integrated with VBA code [25]. The user form or first page asks for input data, mainly body weight (BW) in kg and expected average daily gain (ADG) in g, depending upon which, it will calculate the minimum nutrient requirements, i.e., total DMI in 'Kg', CP in 'g', TDN in 'g'. Then should be selected from the list on the second page from roughages, concentrate, and minerals. Once this information is fed, tool RBT will solve for minimization of cost with DMI, TDN, CP as constraints. The tool RBT followed the following linear programming model [26]. (see eq (8))

Objective Function:

$$Minimize\, z = \sum_{i=1}^{n} c_i x_j \tag{8}$$

Subjected to constraints.
DMI

$$\sum_{i=1}^{n} a_i x_j = b$$

TDN & CP:

$$b_{min} \leq \sum_{i=1}^{n} a_i x_j \leq b_{max}$$

Feeds:

$$0 \leq x_j \leq c_{max}\; j = 1, 2, 3 \ldots n$$

Where c_i is the cost of each feed ingredient, x_j represents the number of feeds, a_i represents the composition of DM, TDN and CP in all feeds, b_{min} and b_{max} are the minimum and maximum requirement of DMI, TDN and CP, c_{max} is the maximum limit for each feed.

3. Results and discussion

Ration balancing tool for growing goat – a Microsoft Excel VBA based software can calculate the nutritional requirements for animals, such as dry matter intake (DMI), crude protein (CP), total digestible nutrient (TDN), and metabolizable energy (ME), for which equations are derived by using common factor method based on the data of ICAR (2013). The table values of nutrients as per ICAR (2013) and calculated values as per software have high R^2 values (DMI-0.989; TDN- 1; CP-0.999) shown in **Figures 2–4**.

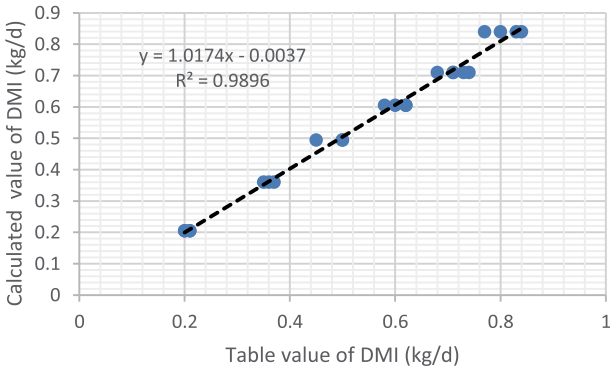

Figure 2.
Comparison of table values of DMI (kg/d) as per ICAR (2013) and calculated values as per software having high R^2 values.

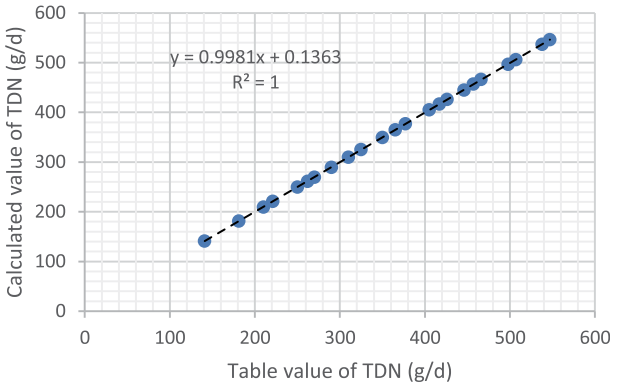

Figure 3.
Comparison of table values of TDN (g/d) as per ICAR (2013) and calculated values as per software have high R^2 values.

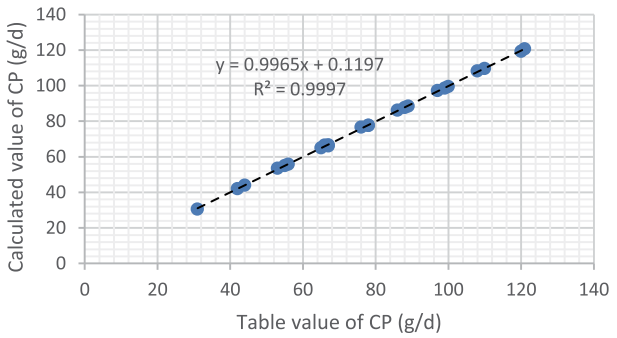

Figure 4.
Comparison of table values of CP (g/d) as per ICAR (2013) and calculated values as per software have high R^2 values.

Once the nutrients requirements are found, the application asks the user to select the available feeds which are listed in the application. Then it will provide a balanced ration using LP model [27]. The expert nutritionist has examined the created application in NIANP, and the results of some specific animal categories (Goat 1 and Goat 2) are given in **Table 3**. The developed RBT will find the least-cost ration with the consumer selected feed without breaching the nutrient requirement shown in the sixth column of **Table 3**.

Animal Details	Nutrient Requirement	Feeds Selected	Suggested feed quantity with price and total cost		Nutrients from feed
Weight (Kg): 15 ADG (g/d):75	DMI(Kg):0.5 CP (g):77.74 TDN(g):349.5 ME(Mcal):1.24	Name	Quantity	Cost (Rs)	DMI(Kg):0.54 CP (g):77.74 TDN(g):349.5 ME(Mcal):1.26
		Maize Green fodder	0.04	0.55	
		Multicut sorghum green fodder	0.17	0.55	
		Ragi straw (dry)	0.03	0.24	
		Wheat	0.22	3.91	
		Soya DOC	0.09	3.87	
		Concentrate Mix Type 1	0.08	2.20	
		MM	0.01	0.61	
		Salt	0.01	0.04	
		Total	0.63	11.97	
Weight (Kg): 20 ADG (g/d):100	DMI(Kg):0.60 CP (g):99.51 TDN(g):444.76 ME(Mcal):1.58	Multicut sorghum green fodder	0.2	0.67	DMI(Kg):0.67 CP (g):99.52 TDN(g):444.76 ME(Mcal):1.61
		Maize	0.14	3.18	
		Wheat	0.16	2.88	
		Soya DOC	0.12	5.26	
		Concentrate Mix Type I			
		MM	0.01	0.75	
		Salt	0.01	0.05	
		Total	0.73	15.48	

Table 3.
Feed formulation for two different goats based on DMI, CP, TDN and ME by RBT depending on the weight and average daily gain.

As per **Table 3**, It is observed that two different categories of goat listed for validation, DMI, CP, TDN and ME criteria, are determined depending on both the weight and average daily gain as in the second column of **Table 3**. The developed RBT will find the least-cost ration with the consumer selected feed without breaching the nutrient requirement shown in the fifth column. To find the optimal solution, the RBT uses the Excel solver. The Excel Solver is efficient in obtaining feasible solutions nonlinear model for goats and increased daily gain and milk yield [28]. The nutrients TDN and CP required for growing goat according to Mandal, 2005 [29] for goat 1 with the body weight of 20 kg and an ADG of 75 g are 351 g and 79 g, respectively, for goat 2 with the body weight of 20 kg and 100 g of ADG, the requirements are 446 g and 100 g respectively. The nutrients TDN and CP calculated by developed application for Goat 1 are 349.5 g and 77.74, and for Goat 2 are 444.7 g and 99.51 g. There is a very small difference 1.5 g (0.4%) and 1.26 g (0.3%) in TDN and CP for goat 1, for goat 2 TDN and CP difference is 1.3 g (1.6%) and 0.49 g (0.49%) between RBT and Mandal et al. (2017). The required nutrients for two different goat categories and calculated by balanced ration are shown in **Figures 5** and **6** for goat1 and 2, respectively.

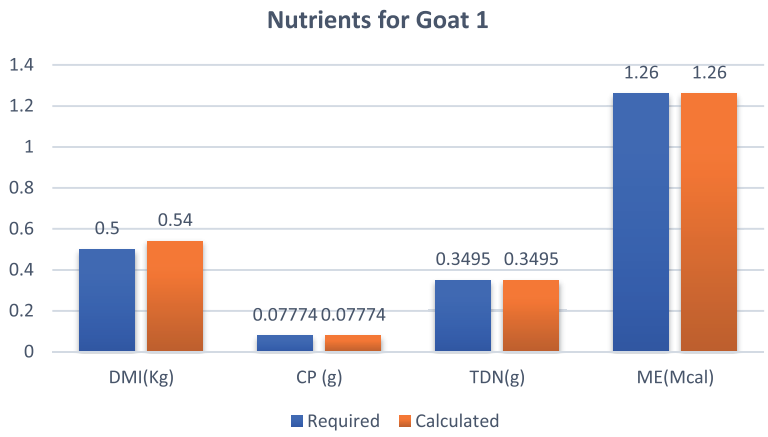

Figure 5.
Graphical representation of required vs. calculated nutrients by balanced ration for goat 1.

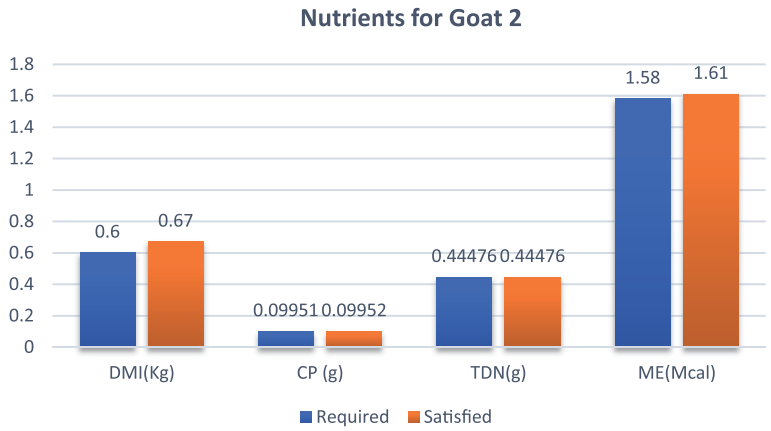

Figure 6.
Graphical representation of required vs. calculated nutrients by balanced ration for goat 2.

From the above studies and evaluation, it can be confirmed that the calculated values for DMI, TDN and CP are almost equal to the values of ICAR (2013). A Ration Balancing Tool (RBT)" is developed using Excel VBA, which gives a balanced ration for the goats with the available ingredients that satisfy all the nutrient requirements. Many software programs are available to customize ration for the lowest cost [30]. However, scanty applications are available for goat least-cost ration formulation. This study explains how the application is exceptional and more efficient and convenient compared to all other software programs, most of which are not user-friendly, and farmers must rely on expert assistance to implement it. For the commercial business reason, many software programs are developed for the client, wherein small dairy farmers still have to rely on specialists for optimized rationing. This tool is very simple to execute and user friendly. It is designed to determine the nutritional requirement of goats, depending on their weight and daily gain and to optimize goat ration at least cost.

3.1 Steps followed for formulation

Step1: once the excel sheet is open, page 1 of the user form will appear as shown in **Figure 7**. This page contains three tabs '*Introduction', 'Methodology' and*

Figure 7.
Screenshot of the first page of developed VBA application showing the introduction, methodology and help sections.

'Help'. Detailed information on goat feed formulation is given in the 'Introduction' tab. The procedure to use the application is given step by step in the 'Methodology' tab. How to add excel options to the applications is mentioned in the 'help' tab.

Step2: Clicking the NEXT button, page 2 will be displayed as shown in (**Figure 8**) where users are required to enter input data such as body weight (kg), expected average daily gain (g) of goat. Depending on the input data, the tool RBT calculates the DMI, TDN and CP required for a particular goat.

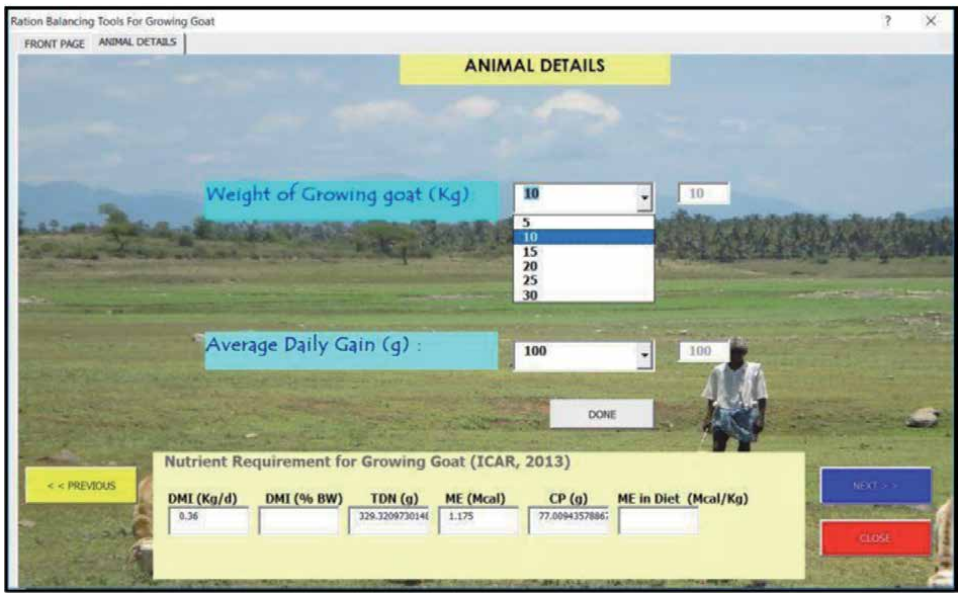

Figure 8.
Screenshot of the second page developed VBA application where the user can input the basic data.

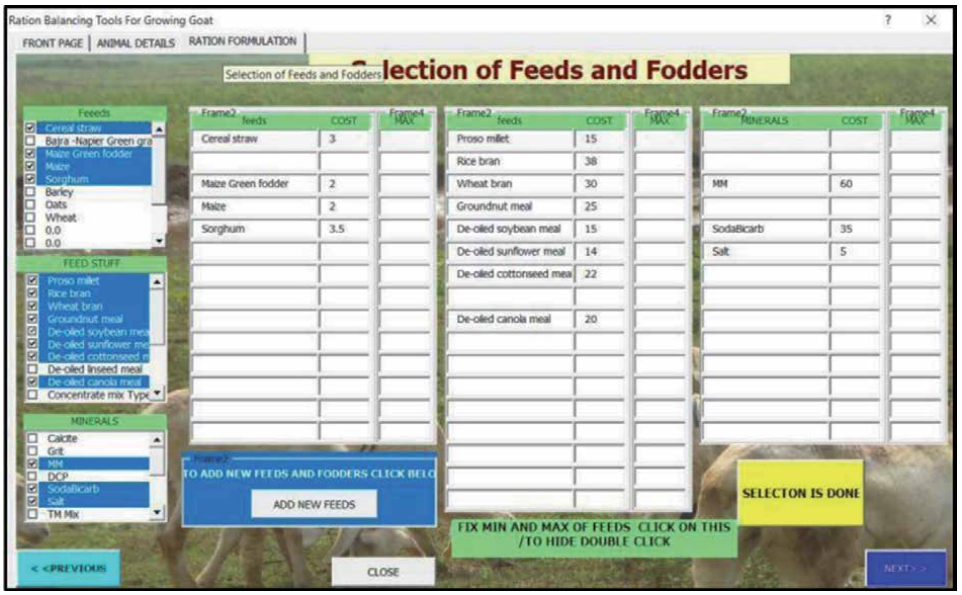

Figure 9.
Screenshot of the third page developed VBA application where users can select feeds from roughages, concentrate and minerals.

Step3: Users must select feeds from roughages, concentrate and minerals on page 3 as shown in (**Figure 9**). The option is given to fix the minimum and maximum feed selected. If not, it will take standard values set by the tool. Users can also add new feeds depending on the availability in the subpage shown in (**Figure 10a**).

Step4: After the selection of feed has been completed, the tool RBT will provide an optimized ration cost to satisfy all nutrients at the minimum cost. If any nutrient requirement has not been met, the app will ask for feed refining where the user has to reselect the feed, this case is shown in (**Figure 10b**).

Step5: For the final output, the user must click on the 'Go to Result'. It opens excel sheet where the user can find.

1. Single goat ration cost.

2. Cost of 100 kg ration on dry matter basis.

3. Ration cost for the number of goats available.

Printouts can be taken for all the results.

The features of the developed application are as follows, firstly Data Maintenance; if no feed is listed, the feed with nutrient composition can be uploaded by the consumer while selecting the feed. It allows the consumer to reasonably apply the feed available locally and reduce the cost. Consumers can get optimized ration in maximum effective steps by selecting the animal data and picking the feeds and then tapping on the "Solve" icon to get the result as it is user-friendly. For Display and printing, after the solution is found, there is an option for the result to be printed on a fed basis with feed quantity and total DM intake per kg. System requirements are also minimal as All MS Office versions can be used, and no special hardware or RAM is needed. The macros and solver options in VBA reference need to be enabled. The application provides the result in three ways: cost of single goat ration: Here, the total DMI is provided for one goat. This will help the consumer get an optimized ration and provide the right amount of roughage, concentrate, and

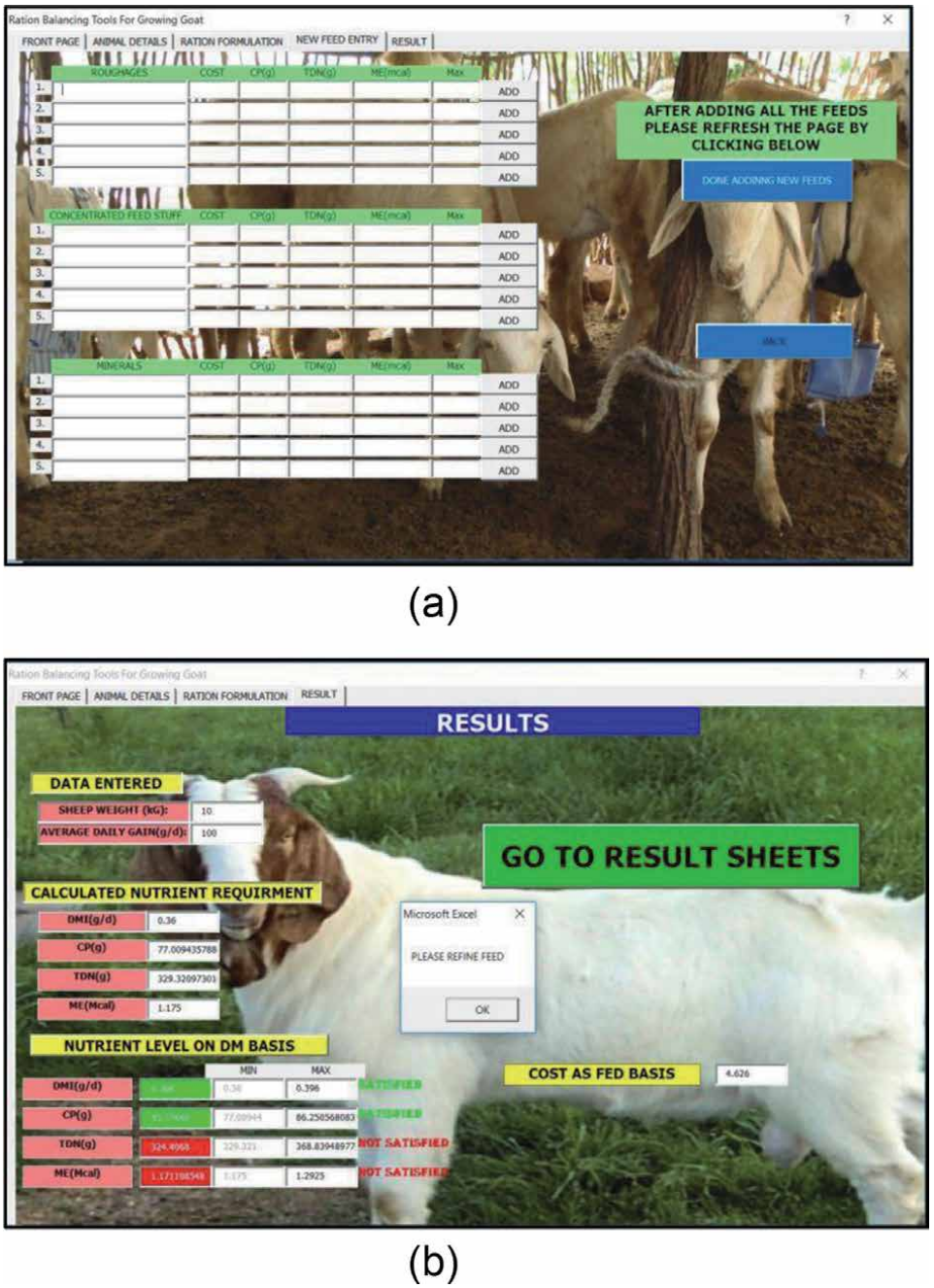

Figure 10.
(a) Screenshot of the sub page developed VBA application where user can add new feed. (b) Screenshot of the fourth page developed VBA application where user can refine the feed.

minerals to be included in the ration. Finally, the price of each feed is given in the result. Cost of 100 kg ration on dry matter basis: Here, it estimates 100 kg at a time, which can be fed to goats at periodic intervals. The amount of roughage, concentrates, and minerals to be added to make 100 kg and its cost will be shown. Ration cost for the number of goats available: Here, the ration will be estimated on a dry matter basis for the number of available goats. The output is given in **Figure 11**.

RATION BALANCING TOOL FOR GROWING GOAT

Name of the company : ICARDA Name of the consultant : Dr.D.Rajendran

Address/Phone No. Phone No: +91-9443900432

Name of the ingredients	Inclusion Level as fed basis	Cost	Name of the ingredients	Inclusion Level as fed	Cost
Roughage			Supplements		
			MM	0.006	0.611
Maize Green fodder	0.037	0.55			
Multicut sorghum green foo	0.165	0.55			
			Salt	0.006	0.043
Ragi Straw (Dry)	0.031	0.24			
Concentrate			Animal Details		
			Weight (Kg)	15	
Wheat	0.220	3.91	ADG (g/d)	75	
			Total ingredient Quantity as fed basis (kg)		
Soya DOC	0.087	3.87	Ingredients	Quantity (kg)	Price (Rs)
			Roughage	0.233	1.34
			Concentrate	0.390	9.98
			Supplements	0.011	0.654
Concentrate Mix Type I	0.083	2.20	Total	0.633	11.974
			Nutrient Content of Formulated Ration		
			DM(Kg)	0.54	
			CP (g)	77.74	
			TDN (g)	349.50	
			ME (Mcal)	1.26	
			Roughage (kg) on DM basis	0.18	
			Concentrate (kg) on DM basis	0.36	

Date: Signature

Figure 11.
Screen shots of output result estimated on a dry matter basis for the number of available goats.

4. Conclusions

This study showed that how the excel VBA can be used to analyzing the nutrient requirement and producing a balanced ration for livestock (goat) are fundamental aspects of reducing goats' feeding cost. Hence Excel Visual Basic Application (VBA) has been developed. Developed 'RBT' for beneficial for dairy farmers, which is based on a linear programming model. The ICAR (2013) table values for nutrients and the software-calculated values have high R2 values (DMI-0.989; TDN- 1; CP-0.999). It can be confirmed from the aforementioned studies and evaluations that the computed values for DMI, TDN, and CP are nearly identical to the ICAR (2013) values. Just by giving the goat's minimum input, the application will calculate the nutrient requirement and the balanced ration at the lowest cost. Adding extra feed allows the user to add the feed available, which can lower the cost. The answer produced by the application is verified by a nutritionist at the National Institute of Animal Nutrition and Physiology (NIANP), Bangalore. Hence, it is concluded that even this application could be used quite effectively by dairy farmers. By understanding the nutrient requirements, the same can be developed for other livestock animals such as cattle, buffalo, and pigs.

Acknowledgements

Raw data were generated at ICAR-National Institute of Animal Nutrition and Physiology. Derived data supporting the findings of this study are available from the author Prof. Rajendran on request. The authors thank all co-workers for their help and cooperation in this trial. Vishal Patil Conceptualized developed Methodology Data Collection, wrote the manuscript, Radha Gupta and Rajendran D Supervised and Validated. Ravinder Singh Kuntal assisted in Writing- Reviewing and Editing.

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Section 2

Genomics and Welfare

Chapter 3

Genetic Control of Wellness in Dairy Cattle

Natascha Vukasinovic, Dianelys Gonzalez, Cory Przybyla, Jordan Brooker, Asmita Kulkarni, Tiago Passafaro and Anthony McNeel

Abstract

With increased selection pressure on milk production, many dairy populations are experiencing reduced fertility and disease resistance. Reducing susceptibility to metabolic diseases, such as ketosis, displaced abomasum, retained placenta, metritis, mastitis, and lameness, has long been excluded from genetic improvement programs, due to low heritability of those traits. However, research has shown that using large producer-recorded data, genomic information, and suitable statistical models can result in accurate genomic predictions for metabolic diseases, enabling producers to select animals with improved disease resistance early in life. Improving wellness in dairy herds not only increases economic efficiency of dairy herds, but also improves overall animal welfare as well as product quality and public perception of dairy farming. This chapter describes the development of genomic predictions for wellness traits in Holstein dairy cows in the United States and presents examples of validation of those predictions in commercial dairy populations in the United States and other countries.

Keywords: dairy cows, wellness traits, genetic evaluation, genomics, validation

1. Introduction

Over the last 50 years, genetic selection to improve milk production in dairy herds has been very successful. In many developed countries, the milk production per cow has more than doubled. About half of that progress can be contributed to genetics [1]. Along with increase in production, dairy farming has become more intensive. While the number of dairy farms is decreasing globally, the average herd size is increasing [2]. Selection pressure for higher yields and intensive farming have been linked to reduced welfare and an increased incidence of many common diseases in dairy cows, mostly due to genetic antagonisms between production and health traits [3–5]. Consequently, dairy cows are becoming less "robust," which have negative consequences for the health and fertility of the modern day dairy cow [6, 7].

Profitable dairy cows are productive, fertile, and mostly "invisible"—they do not require extra attention or intervention to maintain their health through all phases of production. Having a larger proportion of mature cows that are productive and healthy during multiple lactations can enhance profitability of dairy operations. To reach their full potential and longevity, animals need to remain healthy from birth until calving, and then stay healthy and structurally sound, in addition to

regularly calving and producing milk. Dairy animals that experience adverse health events negatively affect herd profitability through increased culling, veterinary expenses, and labor, as well as monetary losses through reduced milk sales [8]. The costs per case of the common dairy cow diseases were estimated to range from $181 for ketosis to $391 for displaced abomasum [9].

Dairy researchers and producers have made progress on providing the best environment for animals to reduce health events through nutrition, management, and housing. Additionally, genetic improvement of health and wellness traits in dairy cows is an attractive option for dairy producers because genetic gains are permanent and cumulative from one generation to another [10].

Genetic evaluation and selection for improved health traits has been lagging compared with selection for production and reproduction in dairy cows due to low heritability of health traits and the lack of centralized recording. Most health events in dairy herds have not been recorded by trained veterinarians, but rather by producers themselves using herd management software. However, research has shown that, given the large amount of data, availability of genomic information, and advanced statistical methodology, it is possible to provide accurate genetic and genomic predictions that producers can use as a tool to improve health and wellness of their herds.

In many countries, including the United States, the most frequently cited reason for not using health data in genetic evaluation of dairy cattle is the lack of a centralized national system to collect health record data. Although most dairy producers record health information of their animals using herd management software, the subjectivity of diagnosis and the user-defined terminology of health events contribute to increased difficulty in using health data in a genetic evaluation due to insufficient accuracy and inconsistency of recording [11]. However, several studies based on large amounts of producer-recorded data have shown that genetic selection for wellness traits can be effective in improving herd health in dairy cattle as long as the recording protocols within a herd are fairly consistent [8, 12, 13].

Genetic evaluation of health traits has a long tradition in countries with routine health data recording. In Scandinavian countries, health traits have been included in breeding programs since the mid-1970s [14]. Currently, over 97% of Norwegian dairy cows are included in the recording system [15, 16]. In other countries, the use of direct health data in genetic evaluation is progressing rapidly. Routine data collection and genetic evaluation for health traits in Germany and Austria started in 2006 [17]. In France, clinical mastitis has been included in routine genetic evaluation since 2010 [18]. In 2014, genetic evaluation for mastitis resistance was introduced for Canadian dairy cows; the evaluation is based on clinical mastitis incidence recorded in the first and second lactation and SCS [19, 20]. In Canada, genetic evaluation for ketosis and displaced abomasum was implemented in December 2016, followed by metritis and retained placenta, hoof health and lameness, and other functional traits in the following years [21].

The advances in molecular genetics and genome sequences have created unprecedented opportunities to select for genetically superior animals and increase the speed of genetic improvement of production, reproduction, and, especially, health traits in farm animals. In March 2016, Zoetis Genetics launched CLARIFIDE Plus, the first commercially available genomic test for wellness traits of dairy cattle. Today, CLARIFIDE Plus provides genomic predictions for 14 health and wellness traits in cows and calves of Holstein and Jersey breeds.

The goals of this chapter are (1) to describe the research leading to the development of genomic predictions for wellness traits mastitis (MAST), metritis (METR), retained placenta (RETP), displaced abomasum (DA), ketosis (KETO), and lameness (LAME) based on large producer-recorded data, genomic information, and

sophisticated statistical methodology and (2) to present examples of studies focused on assessing efficacy of genomic predictions for wellness traits in independent commercial dairy herds in the United States and other countries.

2. Creating genomic predictions for wellness traits

2.1 Data

Phenotypic data have mostly been collected directly from producers upon obtaining their signed permissions. The main source of data was backup files from herd management software DairyComp 305 (Valley Agricultural Software, Tulare, CA), PC Dart (Dairy Records Management Systems, Raleigh, NC), and DHI Plus (DHI Computing Services Inc., Provo, UT). Backup files are processed using internally written scripts, and information on pedigree, production, reproduction, and health events is extracted. Terminology used to record the health events varies across different herds, which was standardized as described [12, 34]. About 300 herds from around the United States have been participating in providing data.

The majority of genotypes used in genomic evaluation have been obtained in the Zoetis genotyping lab. Samples from animals from commercial herds (hair, blood, ear tissue, or semen for males) submitted to Zoetis for genomic testing were analyzed. Upon DNA extraction, genotyping was performed using Illumina BeadArray SNP chips with a number of SNP markers ranging from about 3000 to over 80,000. Raw genotypes were edited following the criteria as described previously [22, 23]. All animals genotyped with lower-density chips (<40,000 markers) were imputed using the program FImpute [24] to a set of 45,245 markers selected based on their call rates and minor allele frequencies that are used in genomic evaluation.

2.2 Trait definition

Health events of interest were extracted from the herd management software backup files. Wellness traits mastitis (MAST), metritis (METR), retained placenta (RETP), displaced abomasum (DA), ketosis (KETO), and lameness (LAME) were considered.

Each wellness trait was defined as a binary event, having a value of one if a respective health event has been recorded at least once during the lactation and zero otherwise. Animal were required to have a lactation record with a valid calving date and lactation number, with a calving interval ranging from 250 to 999 days [23]. Lactations of the same cow without recorded disorders, as well as lactations of all herdmates of an animal without recorded health events, were added as "healthy" records. Phenotype records were checked against the pedigree, and all animals recorded as male as well as those having incompatible birth and calving dates were removed. Records were also removed if an animal in her most recent lactation did not reach an opportunity period, which was defined as a number of days in milk (DIM) by which 90% of all cases of a particular disorder have been recorded, or if the health event was recorded after the highest number of DIM when the occurrence of a disorder was biologically plausible. Animals not reaching the opportunity period were removed from the analysis regardless of whether they were healthy or sick.

Contemporary groups were created by combining the herd, year, and season of calving. Each group was required to have a minimum of 20 lactation records and at least one "sick" and one "healthy" record; otherwise, the entire group was discarded.

2.3 Methodology

Single-step genomic BLUP (ssGBLUP) was the method of choice for creating genomic predictions for wellness traits. ssGBLUP combines all available sources of information–pedigree, phenotypes, and genotypes–into one single evaluation, without the need of post-analysis processing, and incorporating information on genotyped and non-genotyped animals in this method in a straightforward manner [25].

The data were analyzed for each trait separately, using the following threshold model [23]:

$$\lambda = X\beta + Z_h h + Z_a a + Z_p p + e, \tag{1}$$

where λ represents a vector of the unobserved liabilities for the given disorder; β is the vector of fixed effect of parity; parities 1, 2, 3, 4, and 5+ were considered; h is the random effect of herd, and year and season of calving, where $h \sim N(0, I\sigma_h^2)$ with the variance σ_h^2; four seasons were defined within each year: Winter (Dec-Feb), Spring (Mar-May), Summer (Jun-Aug), and Fall (Sep-Nov); a is the random animal effect, with $a \sim N(0, A\sigma_a^2)$, where σ_a^2 is the additive genetic variance and A is the pedigree relationship matrix; p is the random effect of permanent environment with $p \sim N\left(0, I\sigma_{pe}^2\right)$, and e is the random residual, where $e \sim N(0, I)$. X, Z_h, Z_a, and Z_p are incidence matrices corresponding to the fixed effect in $X\beta$ and the random effects of HYS, animal, and permanent environment, respectively.

In ssGBLUP, the inverse of the traditional pedigree relationship matrix, A^{-1} is replaced by the inverse of H matrix, which is the pedigree relationship matrix augmented using genotypes [26, 27].

$$H^{-1} = A^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & G^{-1} - A_{22}^{-1} \end{bmatrix} \tag{2}$$

where G^{-1} is an inverse of the genomic relationship matrix and A_{22}^{-1} is an inverse of the pedigree relationship matrix for genotyped animals only. The genomic relationship matrix G was constructed using allele frequencies for each of the 45,245 SNP markers as described in [28]. By using the "hybrid" relationship matrix H, the SNP markers are utilized to better define relationships among all animals in the analysis.

Prior to genetic evaluation, variance components for each trait were estimated using the same data and model, but without including genotype information. Heritability of each trait was expressed as the ratio of genetic variance (σ_a^2) and the sum of all estimated variances:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_h^2 + \sigma_e^2} \tag{3}$$

2.4 Software

All analyses were performed using the BLUPF90 suite of programs created by Prof. Ignacy Misztal and his team at the University of Georgia in Athens (UGA) [29]. First, the data were formatted and renumbered using the program RENUMF90 v. 1.14. The variance components were estimated using the program THRGIBBS1F90 ver. 2.116. The genetic evaluation was performed with a program CBLUP90IOD2 version 3.21, which is appropriate for massive datasets as it uses iteration on data. To accommodate the large number of genotypes, the algorithm for

proven and young animals (APY) was applied [30]. The APY algorithm generates the inverse of the genomic relationship matrix (G^{-1}) indirectly using recursion based on a proportionally small subset of animals (proven or core animals). Only the genomic relationship matrix for the core animals needs to be inverted; then, the elements of G^{-1} for all other animals (young or non-core) are calculated linearly by recursion, which significantly reduces the computational requirements [30]. Computational details of the APY algorithm are described in [31, 32]. In our analysis, the core consisted of 25,000 animals selected at random. Each trait was run in a separate process, but with the same model and H^{-1} matrix. The reliabilities of estimated breeding values were approximated using the program ACCF90GS v. 2.54 that implements an algorithm that combines contributions of genotypes, pedigree, and phenotypes [33]. Reliability of estimated breeding values (EBV) is formally defined as the squared correlation between true (unknown) and estimated breeding value; in practice, reliability shows how well the estimate represents the true breeding value. Higher values of reliabilities indicate that EBV are more accurate and less likely to change over time, with the addition of new information. Reliability estimates depend on the amount of data available, heritability of the trait, connectedness among the animals in the population, as well as methodology used to estimate reliabilities. In our analyses with a very large number of genotypes, an approximation considers value of the diagonal of the G matrix, g_{ii}, as a proxy for the contribution from genotypes (Daniela Lourenco, University of Georgia, Athens, personal communication, 2016).

2.5 Expression of evaluation results

The solutions for the random animal effect obtained by the cblup90iod program represent raw estimated breeding values (EBV) on the liability scale. To make them easier to interpret, raw EBV for each trait were transformed into probabilities of exceeding the value of the threshold. The threshold value represents the estimated point of transition between the two categories of a binary trait (in the case of wellness traits, the transition from healthy to sick). Threshold values for all traits were estimated from the data. For each animal solution, the probability that a standard normal variable with a mean equal to that solution and a variance of 1 exceeds the threshold was calculated [23]. These probabilities were then transformed into percentages by multiplying by 100, divided by 2 to obtain predicted transmitting abilities (PTA), which are defined as a half of EBV, and expressed as the differences from the average of the reference population, that is, a group of animals selected to represent relevant individuals from current commercial herds. Higher values of PTA (or genomically enhanced PTA—gPTA—if the animal was genotyped) represent higher risk of having a disorder. For example, in a reference population with an average incidence of mastitis of 20%, an animal with a PTA for mastitis of 2.5 will have offspring with an estimated 22.5% chance of getting mastitis during lactation. Animals' genetic merit for wellness traits is reported as standardized transmitting abilities (STA) [34] where;

$$STA = \left(\frac{gPTA - \mu}{\sigma} \times (-5)\right) + 100 \tag{4}$$

where μ and σ represent the mean and the standard deviation of gPTA, respectively. Therefore, a value of 100 represents the average expected disease risk, with animals at 95 or 105 being one standard deviation away from the mean. For wellness traits, larger STA are more desirable for all traits, because they represent lower expected average disease risk. Selecting for a higher STA is expected to result in reduced incidence of the respective disease (**Figure 1**).

3. Genomically enhanced PTA and STA

Table 1 shows the number of phenotypic records, the number of animals with phenotypic records, mean and standard deviation of the incidence, and the estimated heritability of wellness traits. The number of records for cow wellness traits ranged from about 3.2 million for KETO to almost 5.8 million for MAST. Large differences in the number of records available for individual traits were caused by variations in recording among the farms. The mean incidence of the disorders in our analysis varied from 2.6% for DA to 16.7% and 29.1% for LAME and MAST, respectively, indicating that MAST and LAME are the most common health problems in dairy herds.

The estimated heritabilities for wellness traits were in the narrow range from 0.079 (LAME) to 0.112 (RETP) and were comparable to those reported previously based on studies using similar data and methodology [8, 12]. Heritabilities under 10% are generally considered low, due to proportionally large effects of the environment and not to the lack of genetic variability within the population. Traits with low heritabilities require more data to produce accurate estimates of animals' breeding values.

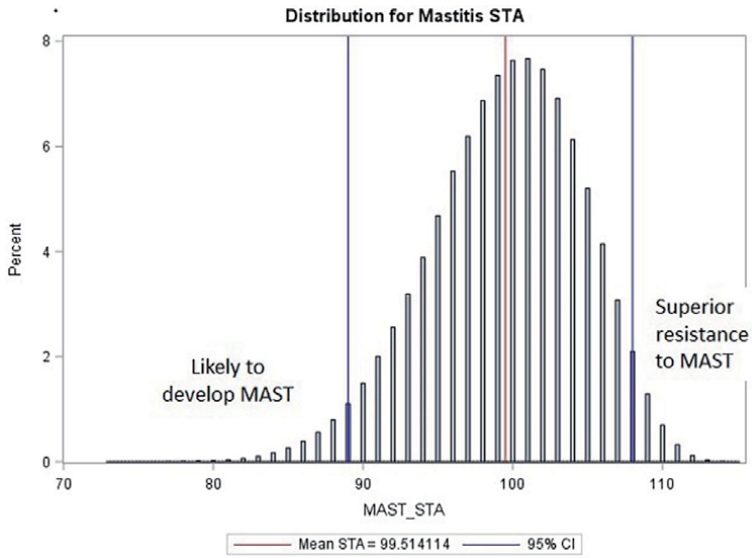

Figure 1.
Distribution of STA for MAST for all animals in the analysis. Animals with extremely low STAs are more likely to develop MAST. Animals with extremely high STAs are considered more resistant to MAST [Dianelys Gonzalez, personal communication, 2021].

Trait	No records	No animals	Mean	SD	Heritability
MAST	5,768,760	2,770,872	0.291	0.454	0.097
METR	4,865,943	2,435,542	0.100	0.300	0.090
RETP	5,505,269	2,714,416	0.050	0.218	0.112
DA	4,489,831	2,262,183	0.026	0.158	0.089
KETO	3,221,467	1,735,818	0.057	0.232	0.081
LAME	4,336,602	2,247,900	0.167	0.373	0.079

Table 1.
Basic statistics and the estimated heritability of wellness traits [Dianelys Gonzalez, personal communication, 2021].

Trait	gPTA				STA				Reliability			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
MAST	−0.543	4.49	−14.10	22.61	99.5	5.1	73	115	50.8	5.44	0	99.8
METR	−0.903	3.02	−9.31	20.75	101.4	5.0	66	115	50.0	5.53	0	99.7
RETP	0.111	2.71	−7.80	18.70	99.8	4.9	66	114	51.4	5.44	0	99.7
DA	−0.249	2.85	−6.77	24.03	100.0	4.5	62	110	46.1	5.52	0	99.7
KETO	−0.964	2.55	−7.05	18.83	101.7	4.8	65	113	46.5	5.64	0	99.6
LAME	0.395	3.73	−10.80	22.19	99.3	5.1	70	115	47.8	5.66	0	99.7

Table 2.
Statistics of gPTA, STA, and reliabilities for wellness traits for genotyped animals (n = 1,512,546) [Dianelys Gonzalez, personal communication, 2021].

Table 2 shows descriptive statistics for gPTA, STA, and reliabilities for all genotyped animals (n = 1,512,546) in the current genetic evaluation. The average values of gPTA and STA were close to zero and 100, respectively, as expected. The variation of gPTA, expressed by their standard deviation and range, reflects the heritability of the trait and the incidence of the disorder. Traits with higher heritabilities and incidence (MAST, LAME) show higher amount of variation in gPTAs. Broader range of gPTA is preferable because it enables better segregation of animals of different genetic merit for wellness traits. Reliabilities for all traits averaged around 50%, but ranging from 0 to over 99%. The reliabilities reflect both the amount of data and the heritabilities of the traits. Very high reliabilities were obtained for bulls with large numbers of phenotyped daughters. A small number of genotyped animals had reliabilities equal to 0. Zero reliabilities for genotyped animals are not expected unless an animal belongs to a different breed or is poorly connected to the population and has an extreme value of the diagonal of the genomic relationship matrix. Animals with zero reliabilities were either crossbreds registered as Holsteins or Holstein animals from unrelated populations from other countries or their offspring without genotyped ancestors or relatives in our data.

4. Validation of genomic predictions for wellness traits

Genomic prediction for wellness trait obtained at young age is considered a useful tool for selection and management for genetic progress and to assist with culling and breeding decisions in the existing herd. Genetically better heifers and cows can be bred with sexed semen, whereas genetically inferior animals can be sold for beef early on or bred with beef semen [35]. It is best practice for any genetic evaluation to assess the effectiveness of the genetic estimates to predict performance of the evaluated animals. For that matter, we conducted a validation study to determine the effectiveness of the wellness trait genomic predictions in US Holstein cows in an independent population of animals [34].

The study involved 11 large dairy herds distributed across the major dairy-producing regions of the United States. One of the criteria for including herds in the study was that they did not provide phenotypic data for the development of genomic predictions for wellness traits. This was important in order to mimic the experience of new customers who decide to genomically test their animals.

Tissue samples from 2875 animals from the 11 herds were genotyped (Zoetis Genetics, Kalamazoo, MI) after which their genotypes and pedigree information were included in the genetic evaluation for wellness traits. gPTA and STA were obtained

for six wellness traits—MAST, METR, RETP, DA, KETO, and LAME. Wellness trait predictions (STA) were used to rank and assign animals to 4 quartiles—genetic groups, for each trait (bottom 25, 26–50, 51–75, and top 25%). Animals were ranked within herd to account for the lack of independence between animal and herd.

Statistical analysis was performed using a GLIMMIX model with a binomial distribution in SAS (version 9.3, SAS Institute Inc., Cary, NC; SAS, 2011). The statistical model included the fixed effects of genetic group, lactation, and age group at the beginning of the study. Herd and animal nested within herd were included as the random effects. The marginal means (incidence) and odds ratios were obtained. The average cost per animal associated with each case of an adverse health event was calculated as a product of the estimated marginal mean and the previously published cost estimate per case of a health event [34].

Table 3 shows the average incidence (marginal means) for the four genetic groups—quartiles—based on gSTAs, the estimated average costs of disease per animal, and the odds ratio compared to the best quartile. The differences in disease incidence between the top and bottom quartiles were 2.9% for retained placenta, 10.8% for metritis, 1.1% for displaced abomasum, 1.7% for ketosis, 7.4% for mastitis,

Trait	Genetic group	Incidence (marginal mean, %)	Disease cost per animal ($)	Odds ratio
MAST	Bottom 25	15.9	33.63	2.03
	26–50	11.2	23.65	1.35
	51–76	11.1	23.32	1.33
	Top 25	8.5	18.00	—
METR	Bottom 25	23.6	70.92	2.10
	26–50	18.5	55.47	1.54
	51–76	19.1	57.42	1.61
	Top 25	12.9	38.58	—
RETP	Bottom 25	4.5	9.30	2.94
	26–50	3.3	6.88	2.15
	51–76	2.5	5.10	1.58
	Top	1.6	3.26	—
DA	Bottom 25	1.1	5.58	17.05
	26–50	0.5	2.32	7.13
	51–76	0.1	0.64	1.95
	Top 25	0.1	0.35	—
KETO	Bottom 25	3.2	3.75	2.20
	26–50	2.5	2.87	1.67
	51–76	1.7	1.97	1.14
	Top 25	1.5	1.73	—
LAME	Bottom 25	11.4	20.23	1.58
	26–50	8.7	15.40	1.17
	51–76	8.6	15.28	1.16
	Top 25	7.6	13.37	—

Table 3.
Results of the analysis of genetic groups for wellness traits in the validation animals [34].

and 3.9% for lameness. The differences in marginal means by genetic groups (disease incidence) translate into appreciable differences in expected economic costs.

4.1 Validation of genomic predictions for wellness traits in other countries

To date, demonstration studies for the wellness traits have been conducted in multiple countries using similar methodology as described in [34] (Anthony McNeel and Fernando Di Croce, Zoetis Genetics Technical Services, personal communication, 2021). In 2020, a demonstration study was conducted using 1053 animals across four farms in the United Kingdom [36]. **Table 4** shows disease incidence (marginal means) of the best and worst third (33%) of the animals when animals are ranked by genomic standardized transmitting abilities (STA) and the estimated disease cost per 100 cows. In this study, a 43% relative reduction in the incidence of mastitis was observed between the bottom and top third of cows ranked on the MAST STA. Translated into economic terms, this equates to £38 a cow per lactation. Similarly, a 42% reduction in the incidence of lameness was observed between the bottom and top third of animals ranked on the LAME STA, equating to £13 a cow per lactation.

These observations have important implications for the sustainability of animal agriculture as fewer health events translate into less antibiotic usage. **Table 5** shows the results for antibiotic use for mastitis treatment in the genomic groups (quartiles) when animals are ranked by standardized transmitting abilities (STA) for MAST. The animals in the best genetic group required almost three times fewer the intramammary antibiotic tubes compared with worst genetic group ranking animals.

Another demonstration study using similar methodology as in [34] was conducted in 2019 across multiple European countries (Anthony McNeel and Fernando Di Croce, Zoetis Genetics Technical Services, personal communication, 2021). Over 4000 animals from 29 dairy herds in 7 different countries (France, Germany, Russia, Poland, Spain, Ukraine, and the Netherlands) were sampled for the study. First and second lactation animals that produced a usable genotype, passed breed check and calved within the desired time frame (April 1st to

Trait	Incidence (%)		Economic losses per 100 animals (£)	
	Best third	Worst third	Best third	Worst third
MAST	11.3	22.3	2083	4025
METR	1.2	5.1	307	1293
RETP	3.0	4.8	699	1137
LAME	23.0	38.2	3586	5949

Table 4.
Results of the independent demonstration study conducted in the United Kingdom in 2020 [36].

Genomic Groups	Mastitis STA Average	No. of tubes per group	No. of tubes per cow	Antibiotic use reduction compared to worst 25%
76–100% (Best)	105	178	0.68	−65%
51–75%	101	250	0.95	−52%
26–50%	98	480	1.83	−7%
0–25% (Worst)	93	518	1.96	0%

Table 5.
Antibiotic use for mastitis treatment in the genomic groups for MAST [36].

Trait	STA quartile group	STA means	Disease prevalence (%) (Marginal mean)	Estimated disease cost (€*) per 100 cows
MAST	Worst 25%	91	42.2	7870
	26–50%	98	36.0	6720
	51–75%	102	36.3	6771
	Best 25%	107	32.7	6098
METR	Worst 25%	95	10.8	2854
	26–50%	100	11.4	3037
	51–75%	103	10.0	2663
	Best 25%	107	7.8	2073
RETP	Worst 25%	93	12.1	2202
	26–50%	99	10.1	1832
	51–75%	103	8.4	1533
	Best 25%	107	6.7	1212
DA	Worst 25%	93	4.5	1963
	26–50%	98	2.6	1113
	51–75%	102	2.0	873
	Best 25%	105	1.7	739
LAME	Worst 25%	92	17.7	2772
	26–50%	98	17.0	2668
	51–75%	101	15.8	2473
	Best 25%	105	15.0	2351

Table 6.
Summary of results obtained in the validation study conducted in 7 European countries in 2019 (McNeel and Di Croce, personal communication, 2021).

September 30th, 2018) were included in the analysis. The incidence of the respective health events and the costs associated with disease were calculated. **Table 6** contains average STA, disease incidence (marginal means), and the estimated disease cost per 100 cows of the genetic groups (quartiles) when animals are ranked by standardized transmitting abilities (STA).

5. Why do genomic predictions for wellness traits work so well?

The validation studies performed in the US commercial herds as well as in the UK and European herds showed consistent results, regardless of differences in location, herd size, and farm management. Genomic predictions for wellness traits in Holstein have been created using data from US commercial herds and they have been shown to accurately predict performance of the animals in Holstein herds not only in the USA, but also in other countries, in herds that did not contribute phenotypic data for development of genomic predictions. How is that possible?

The Holstein population in the United States is genetically fairly homogeneous. A study of genetic variation on the Y chromosome has revealed that more than 99% of all known Holstein artificial insemination (AI) bulls in the United States can be traced through their male lineage to just two bulls born in the 1950s, Round Oak Rag Apple Elevation (Elevation) and Pawnee Farm Arlinda Chief (Chief) [37]. Therefore, the

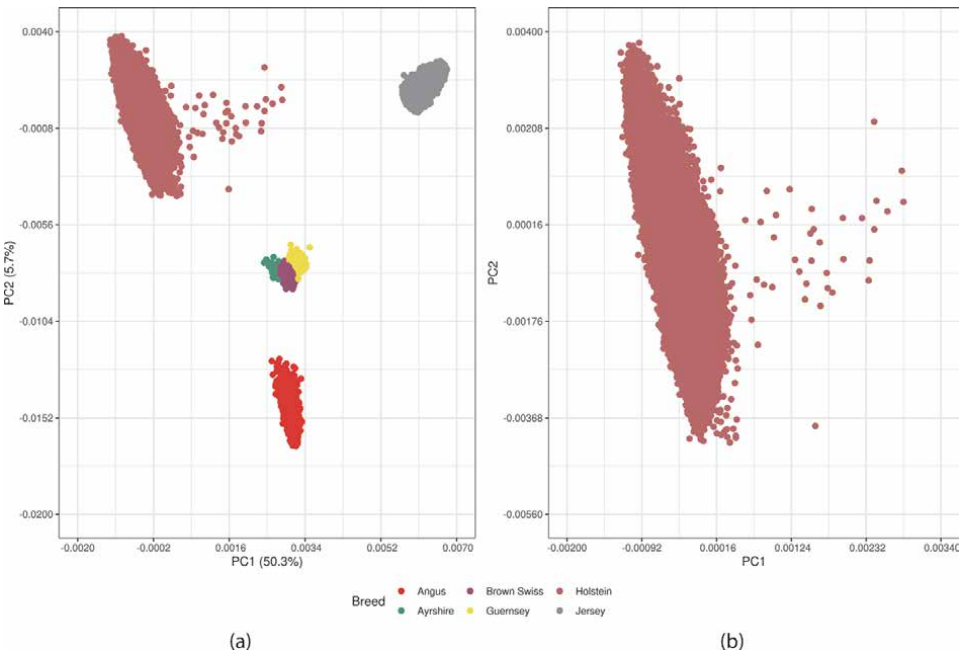

Figure 2.
*(**a**) Principal component analysis of purebred animals; **(b)** magnified Holstein cluster [Tiago Passafaro, personal communication, 2021].*

genomic relationships among all Holstein animals are strong in the United States, as well as in other countries that have imported Holstein genetics (mostly *via* frozen semen). Animals that are well connected to the population used to develop genomic predictions will have accurate predictions for wellness traits even without having their own phenotypes, or phenotypes of their herdmates, in the genetic evaluation.

A small number of animals registered as Holstein may not be well connected to the rest of the population. Crossbred animals or Holstein animals from other countries from populations that did not use Holstein bulls imported from the United States may show loose relationships to the rest of the population, which results in poor predictions and low reliabilities of wellness traits gPTAs, even if the animal has a high-quality genotype in the evaluation. **Figure 2(a)** shows the population structure characterized by principal component analysis (PCA) of purebred animals distributed across the first two principal components, obtained using about 40,000 SNP markers. Breeds included in the analysis were Holstein, Jersey, Brown Swiss, Ayrshire, Guernsey, and the beef breed Angus. It is clearly visible that the individual breeds form distinct clusters, with the Holstein cluster being the largest (due to the largest number of Holstein genotypes in the analysis). However, when magnified (**Figure 2(b)**), the Holstein cluster shows several outliers, that is, animals that fall outside the main cluster, likely due to mild crossbreeding with Jersey. The genomic predictions for wellness traits for those animals may be less accurate than the predictions for animals within the main cluster, due to their poor connection to the rest of the Holstein population.

6. Conclusions

This chapter describes the development of genomic predictions for wellness traits in US Holstein cattle using large producer-recorded data, genomic

information, and sophisticated statistical methodology designed to handle large amounts of phenotypic, pedigree, and genomic data. Genomic predictions for wellness traits have been successfully validated in commercial herds in the United States, UK, and several European countries. These results indicate that genomic data of young calves and heifers can be used to effectively predict future health performance as long as the target population is genetically connected to the population used for developing those predictions. Improving health traits, commonly referred to as functional or wellness traits, through direct genetic selection presents a compelling opportunity for dairy producers to help manage disease incidence and improve profitability when coupled with sound management practices. Genetic selection for improved wellness traits will result in a permanent and cumulative improvement of herd health, as opposed to temporary relief achieved using antibiotics, vaccinations, and other management interventions. Including genomic predictions for wellness traits in an index, along with existing predictions for other economically relevant traits, could provide dairy producers with a more complete tool for selecting potentially most profitable animals.

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Section 3

Production Systems

Chapter 4

Leveraging Livestock Production Systems for Human Nutrition in Developing Countries

Ditty Maria Dominic and Hans Ram Meena

Abstract

Livestock farming is a key sector that promotes socio-economic development in developing countries with around 600 million smallholders relying on it for livelihood. The multi-functionality of livestock production in the livelihoods of smallholders, from an income and input generating asset to a source of food and nutrition, is well known but less acknowledged. Though the concept of leveraging agriculture for nutritional goals is gaining importance, the evidence on the impact of nutrition-sensitive agriculture interventions is sparse particularly in one of the major subsectors in agriculture like livestock. The current chapter discusses the potential of livestock farming systems to tap nutritional outcomes in developing countries where multiple forms of malnutrition are highly prevalent due to over-reliance on starch-based diet and other socio-economic and cultural factors. Thus, the chapter highlights the importance of animal source foods (ASF) in human nutrition, the pathways linking livestock and nutrition, the sustainability issues related to livestock production systems, and the way forward to exploit these systems as a tool for tackling malnutrition in the developing world.

Keywords: livestock, nutrition, smallholders, developing countries, nutritional security, malnutrition

1. Introduction

Livestock, the world's fastest-growing agricultural subsector is also a key sector that aids economic and social growth in developing countries. It has been a major livelihood strategy for more than 600 million smallholder farmers in these countries [1]. They consider livestock keeping as an important asset that not only contributes to income generation but also accounts as a major source of animal source food (ASF), agriculture input like manure, traction, and also a safety net during emergency situations or lean agricultural seasons. Along with livelihoods, livestock supports the food and nutrition security of around 1.3 billion smallholders and contributes about 40% of the global value of agricultural output in developing countries [2]. Nutrition-sensitive agriculture interventions and studies have gained momentum in the past years though the impact evidence is scarce particularly in the livestock subsector. Various systematic literature reviews have acknowledged that livestock interventions have not yet effectively considered nutritional outcomes and that the number of studies assessing impact is even scarcer [3–5]. Considering its contribution to global gross domestic product (GDP), as well as being constituted by five of the 10 highest

value commodities, the livestock sector can be used as an efficient tool for poverty elimination and achieving food and nutrition security in developing countries [6, 7]. Moreover, half of the world's poor people rely on the sector for subsistence, as well as income, insurance, and food, hence increasing food security and nutrition without emphasising on livestock would be a missed opportunity [8, 9].

Around 1 billion people, which accounts for one seventh of humanity, in Africa and Asia depend on livestock production and marketing for livelihood [10]. Further, the potential of livestock production systems to achieve food and nutritional security is evident from the fact that 34% of protein and 18% of calories consumed globally is supplied from livestock production [11], and livestock production allows food production on 57% of the earth's land that cannot be used for crop production [12]. Livestock can act as a transformative tool in the lives of the poor. It is a potential solution to tackle food and nutritional insecurity as well as poverty. Income generated from livestock can provide education, health, and other serviced necessary for the enhanced livelihood of the poor. But the challenge is to balance the positive and negative aspects of livestock production systems so that the advantages are fully realised while the negative effects on health and the environment are mitigated. This chapter elaborates on the potential of livestock for achieving improved human nutrition outcomes and its linkages to nutritional and health status by doing a critical analysis of the available literature on the topic.

2. Methodology

Considering the relevance of the topic, a thorough analysis of previous studies and works was done to obtain a comprehensive summary on the topic. The literature review surveyed research articles, books, short communications, conference papers, and other internet sources on the topic.

3. Animal source foods (ASF) and human nutrition

The main direct link of livestock to nutrition is through animal source foods (ASF), which are the inimitable source of high-quality proteins as well as bioavailable critical vitamins and minerals. This can be imperative in the diets of people in developing countries, which typically is carbohydrate dense and nutrient deficient and as evidence, these countries have the highest prevalence of anaemia in women [13]. The deficiency of micronutrients of which the ASF are abundant, like vitamin A, iron, iodine, zinc, B12, and folic acid, are very scarce in the diets of children and pregnant women and this causes poorer growth, cognitive disability, perinatal problems, and an increased risk of incidence and death. [14, 15]. Consumption of even relatively small amounts of animal-sourced foods contributes substantially to ensuring dietary quality [16]. For example, a woman would only have to eat about one eighth and over one third time as much liver and beef respectively as spinach to meet her daily iron needs [17]. Studies also report that animal-sourced diets are indeed the finest sources of essential nutrient-rich diet for children aged 6 months to 1 year [18]. Compared to plant foods, ASF provides more bioavailable vitamin A, vitamin D3, iron, iodine, zinc, calcium, folic acid, and necessary fatty acids, as well as higher quality protein [19, 20].

4. Evidence on the impact of ASF on human nutrition

Studies conducted worldwide provide strong evidence of the potential of ASF in improving nutrition especially in low-income and developing countries.

Stunting and underweight which are indicators of chronic and acute malnutrition were reduced by 47–74%, respectively, in a randomised controlled experiment in Ecuador [21]. A study on children aged 6 months to 1 year from 49 countries found strong links between stunting and a generic ASF consumption indicator and concluded that consuming ASF was strongly related to child stunting [22, 23]. Supplementation of basal diets of Kenyan schoolchildren with small amounts of meat or milk increased their cognitive skills, leadership behaviour, physical activity, and initiative [24]. Iron-containing complementary foods like meat are especially important among infants who have insufficient iron stores or inadequate intake, as concluded in a recent systematic review [25]. Deficiencies of nutrients like vitamin B12, vitamin A, iron, zinc, docosahexaenoic acid, and iodine, which are critical for neurological development are present in ASF, have been associated with brain-related disorders, including low-intelligence quotient, autism, depression, and dementia [26]. Dairy consumption improves bone health during childhood and adolescence and reduces the risk of osteoporosis and type 2 diabetes [27]. Though studies have clearly reported strong evidence on the positive impact of ASF on human nutrition, its consumption is not adequate in developing countries. Hence, more effort needs to be taken to promote its consumption.

5. Consumption pattern of ASF in developing countries

In developing countries, people consume a low amount of ASF which leads to developmental abnormalities, anaemia, decreased cognitive abilities, and weaker motor development as ASF is the only natural source of vitamin B12 [28]. According to WHO, low-income countries consume a lesser amount of ASF than developed nations. Inadequate access to animal-sourced foods is a major problem in developing countries. This is coupled with other factors like unavailability of ASF, lack of awareness about their prominence in the diet; as well as poverty, gender dynamics, taboos, and other socio-cultural factors. Unawareness of the importance of ASF in diets is an issue majorly among rural poor wherein diets are aimed at eradicating hunger rather than meeting nutritional requirements. This scenario is aggravated by the unaffordability of diets by the poor. According to the study conducted by researchers (for example, see [29]) in India, the milk consumption by rich households are around 7 and 3.3 times higher than poorer and poor households respectively. Also, the prices of ASF are higher than plant-based foods making it less affordable for the poor. In Ethiopia, the prices of ASF like egg, meat, and milk increased by 30% in the last decade compared to plant-based foods like tubers, roots, and cereals as per the findings of the study conducted by [30]. Religious and other social taboos further contribute to low consumption of ASF like most of the Hindus in India do not eat beef and Muslims do not eat pork due to religious beliefs. Some believe that if lower caste people eat ASF, animal productivity will decrease [31]. Studies [32] also report that gender bias in food allocation is another undeniable factor in low ASF consumption in developing countries. As per the meat consumption per capita and stunting rate estimates in different countries by OECD (for example, see [33]), there is a decreasing trend in the proportion of stunted children in various countries across the world with increasing per capita consumption of meat and developing countries had low per capita consumption of meat compared to developed countries. Although the ASF food consumption is generally low in developing and other low-income countries when compared to developed ones, studies predict that overall there will be a hike in per capita consumption of livestock products compared to other agricultural products and this change will be more evident in developing countries [34].

6. Pathways linking livestock and human nutrition

The agricultural nutrition impact pathway has been a subject of a study recently, one of the prominent frameworks is put forward by [35, 36]. Pathways specific to livestock and nutrition were put forward by Randolph *et al.* [37], and the risks in the pathways were identified by [38]. Also, Dominguez *et al.* [39] elaborated on a broad conceptual framework (see **Figure 1**) for hypothetical linkages between livestock keeping and human nutrition. According to the below framework, animal/livestock ownership has both beneficial and negative links to human health and nutrition. Role of livestock in sustaining rural economy: Roles and contributions of livestock can be classified into production function which is the direct production of food and non-food items; non-production function which is mainly as input provider; asset function wherein it acts as a source of income in an emergency situation; and sociocultural functions.

6.1 ASF pathway

It is regularly quoted that 'livestock products provide one-third of humanity's protein intake'; this broad estimate, based on FAOSTAT23 data, came from [40]. ASFs also offer a resilient source of nutrition during seasonal or climatic fluctuations in the availability of plant-sourced foods. As ASF are income elastic, the consumption of ASF by rural poor usually is supplied from their own production [41], and some studies done in Africa showed that livestock keepers are more likely to eat ASF than non-livestock keepers [42], although it is not always necessarily own-produced. For any effective intervention in improving nutrition through livestock, it is essential to thoroughly look into the linkage pathways between the two. Considering the positive links between livestock and human nutrition, the major linkage pathways are an increase in income, increased availability and accessibility of ASFs, provision of inputs like manure, traction, etc., and women empowerment. The direct pathway through which livestock contributes to human nutrition is by providing ASFs that

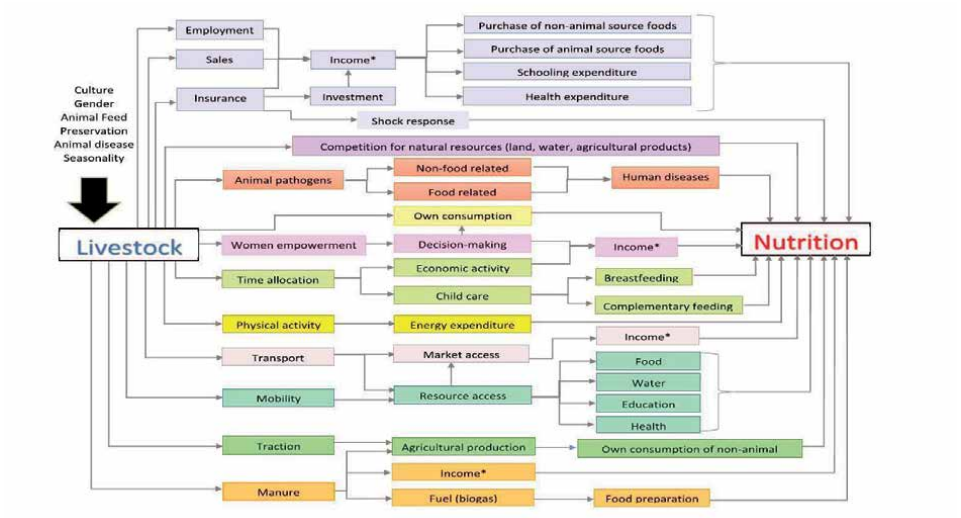

Figure 1.
Diagram of impact pathways through which livestock can affect nutrition. Pathways identified during the workshop 'Livestock, livelihoods and human nutrition', Senegal, November 2014. Adapted from Dominiguez et al. [39].

improve the diets of livestock rearing households. The role of ASF in human nutrition and its importance in diets are clearly discussed in the previous sections.

6.2 Income pathway

The contribution of livestock to household income ranges widely, from 2% to more than 33% in a number of developing countries [43]. It is assessed that more than 80% of poor Africans and up to 66% of poor people in India and Bangladesh keep livestock [44]. Thus, livestock as a source of income and employment is one of the other major impact pathways. According to studies [44], ASFs are income elastic, which means their consumption increases with an increase in income. ASFs are normally associated with wealth as higher income households eat more ASF than poor households [45]. Income from livestock can be through sale, employment, and insurance. Livestock is generally called the savings bank on hooves of smallholder farmers. Livestock function as insurance policies and bank accounts in many parts of the developing world [46]. The capitalisation of underutilised family labour, assets like manure, draft power that can be either used or sold also contributes to the income in an indirect way. As per the data of the international labour organisation, the livestock sector contributes to 60–70% of employment in developing countries, particularly in Asia and Africa. Thus, being a potential sector that provides employment to a larger section of society livestock sector enhances the income provides security in emergency situations through insurance, which eventually improves the diets of people by positively influencing their dietary choices and other medical and educational needs.

6.3 Women empowerment pathway

Another major pathway from livestock to nutrition is through women empowerment. Gender bias in food and nutrition security is a much-researched topic recently. Women in households are more prone to food and nutrition insecurity than men, which ultimately causes them nutrient deficiency and non-communicable diseases like anaemia [47]. Studies show that, globally, malnourished people decrease by 100–150 million with women's access to inputs and services. Women's income is found to be spent on their family's nutrition [48]. In developing countries, poultry and small ruminants are often owned by women, the income from which makes them more empowered and their families more nutritionally secure [49]. Women play a critical role in livestock rearing in developing countries, they are often the ones responsible for feeding and care and are the guardians of livestock diversity. Improving women's access to inputs and services has the potential to reduce the number of malnourished people in the world by 100–150 million [50, 51]. Being a non-seasonal source of income and considering the higher involvement of women in this sector, livestock keeping has undisputable potential to empower women as it gives them access to resources and choice of better diets and health practices [52]. Thus, women's empowerment through livestock is important for their as well as future generations' empowerment in nutrition.

6.4 Adverse pathways

Livestock can also have an effect on food security by spreading diseases to people via carriers such as biting flies and contaminated animal source foods; these diseases reduce people's productivity by limiting their capacity to produce food or labour to earn money to buy food. With 13 major zoonotic illnesses killing 2.2 million people per year, mostly in low- and middle-income countries [53], livestock has a considerable impact on human nutrition and health. Livestock keeping may

also increase the probability of zoonotic disease or indirectly affect human health through contamination of water bodies which are the negative influences. Some of the most common diseases in humans, such as measles, influenza, and diphtheria, have always been transferred by the animals they owned, a phenomenon is known as the 'fatal gift of livestock' [54]. Environmental enteric dysfunction (EED)—an important risk factor for stunting—is associated with chronic inflammation in the intestines of young children and asymptomatic infections by diverse enteric pathogens including those present in livestock manure [55].

In developed and developing countries alike, antimicrobial-resistant pathogens, often associated with the intensification of production systems, are commonly found in animals, animal food products, and agro-food environments [56]. Additionally, women are more at risk of zoonotic diseases because they are more likely to be exposed and maybe particularly vulnerable [57]. Much of the existing burden and danger may indeed be reduced if better disease control technology and institutions were used, as well as investments in zoonosis control innovations. Increased consumption of animal-derived foods, on the other hand, may increase the risk of food-borne illness or the emergence of chronic diseases, both of which would have a detrimental influence on human nutrition. Overconsumption of foods of animal origin can have a negative influence on human health and well-being, affecting both entire societies and households. Overeating fatty red meats and hard cheeses, which contain more saturated fats, can cause cardiovascular disease, while excessive eating of processed meats like bacon and ham has been linked to several cancers [58]. Increased intake of high-energy meat, milk, and eggs contributes to global obesity as well. The physical activity in livestock rearing is much higher causing higher energy expenditure and ultimately affecting nutrition and health. Other than this competition of natural resources like land, water, and other agricultural products are other factors that can negatively influence nutrition. Recently the lack of sustainability of livestock production systems is also been widely discussed considering the ill effects on the environment. But facts need to be checked before de-promoting livestock production and consumption of ASF based on the sustainability criteria. This is discussed in the below section.

7. Livestock production and sustainability

The sustainability of livestock production systems is questioning the potential of livestock production systems for nutrition in recent times. Researchers have called for reducing the environmental cost per unit of livestock considering the current damage caused by the drastic growth in livestock production [59, 60]. Livestock manure produces 65% of human-related nitrous oxide, which has 296 times the global warming potential of carbon dioxide. Livestock account for 37% of all human-induced methane production—and the warming effect of methane is 23 times greater than that of carbon dioxide. Sustainability valuation of livestock food systems is generally based on greenhouse gas (GHG) emissions. But to get a clear picture of the GHG emission scenario we need to thoroughly examine the sector-wise contribution to GHG emission globally. The world emits around 50 billion greenhouse gases each year. According to the data of World Resources Institute, over three quarters of emissions originate from energy usage, nearly one fifth from agriculture and land use (which rises to one quarter when we examine the entire food system—including processing, packaging, transport, and retail), and the rest 8% from industry and waste [see **Figure 2**]. GHG emission is 12–18% from the animal source foods production systems wherein the benefits offered by whole livestock production systems in agriculture, nutrition, health, and other sectors are completely ignored. The overlooked fact is that these benefits counterpoise the

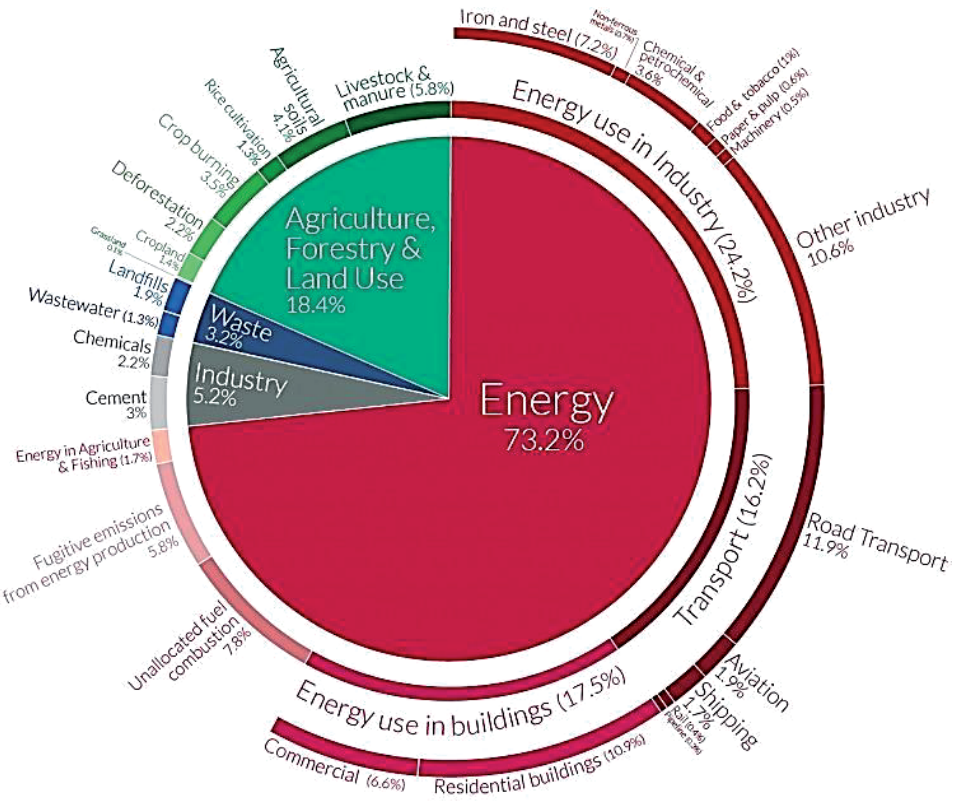

Figure 2.
Sector-wise GHG emissions. Source: Climate Watch, World Resources Institute, 2016.

greenhouse gas emissions, which have declined over the years due to the improved livestock management practices [61].

If we consider the nutrient-rich products derived from livestock production systems which are essential for human health and well-being, the problem of unsustainability like the generation of larger carbon footprints is well balanced [62]. Also, livestock systems contribute to global sustainability by providing various ecosystem services as reported by studies which found that grazing lands has lower greenhouse gas emission or more carbon sequestration than the same land converted for crop production [63] and net accumulation of soil carbon or sink of greenhouse gases was greatest when grassland was converted to silvo-pastures combining trees, forage, and livestock [64]. Furthermore, forage crops make land unfit for cultivation more productive as 57% of forage lands worldwide, are non-cultivable [65]. Also, livestock feeds 86% of products that are inedible by humans like industrial by-products, crop residues, and grasses or fodder, which is then converted into high-value ASF contributing to health and incomes with the bonus of evading environmental pollution from burning or dumping the residues and by-products [66]. Thus, the sustainability of livestock production systems needs to be analysed based on all these facts rather than a one-sided view.

8. Conclusion

Properly managed livestock can play a role in addressing malnutrition. Greater integration between the livestock and nutrition sectors is necessary to ensure livestock livelihoods and animal-sourced foods contribute to fighting malnutrition. The contribution of the livestock sector to a sustainable food system cannot

be overlooked as it converts a significant million tons of useless by-products from agro-industrial sectors into livestock feeds, thus reducing waste and environmental pollution while contributing to food production alongside. These aspects are often overlooked in discussions about livestock and sustainability [67] that received global attention and unfortunately catalysed widely-circulated non-scientific media calling for less ASF consumption in order to save the planet [68]. This highlights the fundamental problem of advocating dietary change towards less ASF consumption [69] —it is an unbalanced view of sustainability that does not adequately address the needs of the most vulnerable. This emphasises the need for significant additional investments in research and development to curtail greenhouse gas emissions from livestock systems, particularly from the ruminant production systems that contribute the most emissions. Infectious diseases at the animal-human interface are highly dynamic and livestock are a major source of zoonotic diseases. Minimal success has been achieved in the control of these infectious diseases in developing countries especially, resulting in a high burden of human gastrointestinal disease [70]. The risks of zoonotic diseases like environmental enteric dysfunction (EED), antimicrobial-resistant pathogens, highlight the need for improved management practices to increase food safety and mitigate disease risks from livestock. Finally, overconsumption of certain ASF may increase the risk of developing chronic diseases such as cancer and diabetes [71]. Thus, while efforts should be made to increase consumption of ASF among the poorest, the general goal needs to emphasise moderation in ASF consumption and adherence to recommended daily intakes [72].

9. Way forward

Though the importance of livestock in the human nutrition domain is a well-established fact, the major attention and focus have been on the adverse effect of livestock production in recent studies, which skews the current discussion. But the other side of the coin is that these studies focus on the livestock production systems in high-income countries that cater the overconsumption there. This neglects global attention on the outlook and necessities of a large number of undernourished people in developing and underdeveloped countries, among whom adequate ASF consumption could prevent malnourishment, health, and development problems. The impact of low ASF consumption on the lives and futures of nutritionally vulnerable people, women, and children, must be considered for the planet's sustainability—a point of view that is sometimes overlooked or underrepresented in scientific studies or heated debates over the effects of livestock production on sustainability. What is also missing is an understanding of how low the consumption of ASF is among the poor, particularly in low to middle income country (LMIC), where starch-based diets are typical. Nutritional, genetic, health, and management measures have been developed by animal scientists to cut greenhouse gas emissions by up to 30%. Hence, one of the important answers for guaranteeing the sustainability of animal production systems is to develop sustainable diets that are lucrative, ethically and socio-culturally appropriate, and ecologically responsible. Consequently, future research on sustainable ASF diets should focus on both animal physiology and farmer behaviours in order to establish a holistic, dynamic, and adaptable conceptual framework [73].

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Section 4

Reproduction

Chapter 5

Importance of Monitoring the Peripartal Period to Increase Reproductive Performance in Dairy Cattle

Ottó Szenci

Abstract

Parallel with the successful genetic selection for higher milk production in Holstein-Friesian cows, a dramatic decline in fertility rates has been observed around the world. Therefore, to achieve an optimum herd reproductive performance, we must focus on the first 100 days postpartum. During and after calving, a cow overcomes a series of physiological hurdles before becoming pregnant. By selecting accurate diagnostic devices and/or methods, such as predicting the onset of calving, monitoring activity and rumination time to determine cows for early treatment of clinical metritis and/or metabolic diseases, long-term measurement of reticuloruminal pH to monitor subclinical acidosis, perform metabolic profile tests to diagnose subclinical metabolic diseases at the herd level, estrous detectors and/or detection aids, on-farm/in-line P4 test to monitor specific events in the postpartum periods, diagnosis of early pregnancy and pregnancy loss using ultrasonography to correctly identify problems and their potential causes to enable these issues are to be rectified. Despite higher milk production, acceptable fertility results can be achieved, even on large-scale dairy farms, if the impacts of the above factors that contribute to reduced fertility can be moderated. The advantages and disadvantages of the different diagnostic methods are discussed to help the dairy select the most accurate method.

Keywords: calving, uterine diseases, metabolic disorders, estrous, artificial insemination, pregnancy diagnosis, diagnosis of pregnancy loss

1. Introduction

Since 1960 due to successful genetic selection of Holstein-Friesian cows for higher milk production, the average milk production in the United States has exceeded 11,000 kg/year. Parallel with this, the reproductive performance of dairy cows declined; however, only the conception rate was considered for the comparison to milk production [1–3]. Others could not confirm this antagonistic relationship between milk production and fertility [4–6]. LeBlanc [5] emphasizes that individual (age at first calving, parity, body condition, and diseases) and herd-level (herd size, nutrition, season, environment, herd/reproductive management, and skilled farm personnel) factors may substantially influence

the production of a dairy farm. At the same time, any shortage in individual or herd-level factors in a dairy farm may increase the average number of days open (calving to conception), the number of services per conception, and the number of cows culled for infertility [2]. It is important to emphasize that reproductive performance in heifers was not affected [7]. It is essential to improve our reproductive management practices to decrease the longer lactations and the number of cows culled for reproductive reasons [7].

Concentrated management activities, especially during the first 100 days following calving, are needed to achieve an optimal calving interval (less than 400 days) with higher milk production per lactation and the birth of more calves [8]. Correct reproductive management can significantly contribute to reducing production costs.

The following diagnostic activities should be pursued during the early postpartum period to achieve or approach the optimal calving interval: prediction of the onset of calving, early diagnosis of postparturient uterine and metabolic diseases, accurate detection of estrus, determining the optimal time for artificial insemination (AI), and accurate diagnosis of early pregnancy and pregnancy losses.

2. Prediction of the onset of calving in dairy cows

One of the essential reproductive management goals is to reduce the number of calving assistances, which may negatively affect the acid-base balance of newborn calves and thus increase the number of stillbirths [9–11] and may subsequently affect the reproductive performance of the dam [12, 13]. Therefore, we must decrease the prevalence of neonatal asphyxia at calving, since instruments suitable for the reliable clearing of respiratory passages and artificial respiration of newborn calves are not yet widely available under farm conditions [14, 15].

In the case of dystocia, we must select the mode and time of calving assistance according to the profitability factors. Before applying traction, we must evaluate the soft birth canal, and it must be expanded nonsurgically or surgically (episiotomy lateralis). With using obstetric lubricants, we must avoid traction of longer than 2–3 min [16] and rib or vertebral fractures due to excessive traction [17, 18]. If prolonged traction is expected, we should perform a Cesarean section to save the calf and prevent maternal birth canal injuries. Previous studies have shown that before selecting the mode of calving assistance (traction or Cesarean section), it would also be essential to measure the acid-base balance of the fetus to be born [19–21]. The routine treatment of newborn calves with severe asphyxia may reduce postnatal calf losses [14, 15]. However, particular attention to the ingestion of sufficient qualities and quantities of colostrum must also be paid [22, 23], since an increased susceptibility accompanies poor colostrum uptake to *Escherichia coli* infections [24, 25].

While it is not possible to eliminate dystocia, adequate management of heifers during the development (adequate feeding, selection of a sire with a negative expected progeny difference for birth weight, or using sexed semen for AI) and close observation of calving heifers and cows are crucial for reducing the prevalence of stillbirth [14]. Since the behavioral signs of calving in some cases are not expressed, it is not easy to recognize the onset of calving, especially on large dairy farms. Inserting a vaginal thermometer into the vagina (e.g., Vel'Phone®) may help decrease the prevalence of stillbirth by sending an alarm about the imminent start of the second stage of labor with the rupture of the allantochorionic sac [26].

In our recent experiment, 241 single calvings were monitored using a vaginal thermometer (Vel'Phone®), which was inserted into the vagina by a vaginal

applicator about 5 days before expecting to calve. The stillbirth rate was 1.7% for heifer and 0.5% for cow calvings, respectively [26]. Similar results were reported by others [27]. Imminent calving can be predicted without false alarms (**Figure** 1) [28], and in this way, it can minimize the time spent on standby by the workers [29].

At the same time, it is essential to mention that in contrast to direct indicators (vaginal thermometers), calving predictors such as Ruminact® HR-tag or Moocall® calving sensor cannot inform about the exact time of calving; however, they can help optimize worker efficiency [29]. It is also essential to avoid birth injuries and infection of the reproductive tract, which may more likely develop in cows with inappropriately timed obstetrical assistance (less than 50 min after amniotic sac appearance) and dystocia [30]. Namely, premature obstetrical assistance may lead to a high prevalence of dystocia, impairs postpartum health of the dam, and poses a potential risk to calf survival [30]. At the same time, Villettaz Robichaud et al. [31] reported that systematic early obstetrical assistance at calving (15 min after the first sight of the calf's two front hooves) that does not present signs of calving difficulties did not adversely affect calves' likelihood of being stillborn, vigor at birth, or transfer of passive immunity. It is essential to mention that obstetrical lubricant was applied liberally to the cow's vagina before performing the exam and providing assistance. Pumping copious amounts of sterile obstetrical lubricant around the fetus before each assisted delivery seems that the target prevalence of stillbirth (1–3%; [32]) can also be approached in the field [33].

3. Early diagnosis of postparturient uterine diseases in dairy cow

The aim of monitoring postparturient uterine diseases such as clinical metritis, clinical endometritis (pyometra), and subclinical endometritis (SCE) in a dairy farm is to diagnose [34–37] and treat them as soon as possible after calving to decrease their negative effects on pregnancy rates, open days, and culling rates, which may increase economic losses in dairy farms [38, 39].

Besides minimizing stress and careful sanitation during calving, cows having dystocia, stillbirth, retained fetal membranes (RFMs), metabolic disorders (hypocalcemia, ketosis), or twins are more likely to contract uterine infections than cows calving normally. Although retained fetal membrane (RFM) is not a disease per se

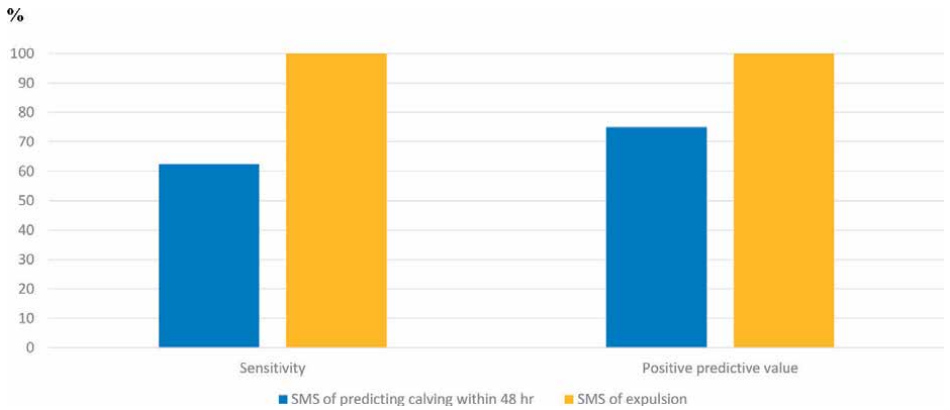

Figure 1.
Accuracy of prediction of calving by an SMS message generated by using an intravaginal thermometer adapted from [28].

[39], its early treatment is greatly recommended to decrease the risk for the development of different uterine diseases.

Clinical metritis and endometritis should be diagnosed and treated as early and as intensively as possible to shorten the conception interval. Recently, new cow programs have been developed based on monitoring cow temperatures each morning for the first 10 (first 13 [40] or 14 [41]) days after calving, thus allowing for early treatment [42]. Monitoring milk production (calving milk deviation of more than 12%) or failure to increase milk yield by at least 4% (primiparous) or 7% (multiparous) per day in the first 20 days after calving [39], rumination time [43–45], cow activity [46, 47] and/or body temperature measured by ear tag, neck collar, vaginal-mounted type biosensors [48], or ventral tail base surface temperature sensors [49] may contribute to the early diagnosis of clinical metritis in the field.

Clinical endometritis can be diagnosed by transrectal palpation, transrectal ultrasonography, manual vaginal examination, vaginoscopy, and/or Metricheck® from Day 21 after calving. In the absence of a gold standard, it seems that vaginoscopy or Metricheck® is preferred as a cow-side diagnostic tool for diagnosing clinical endometritis in the field. Subclinical endometritis (SCE) is defined as an inflammation of the uterine endometrium that can be detected by histology (biopsy) or cytology (samples collected by uterine lavage, cytobrush, or cytotape techniques) in the absence of purulent material in the vagina [37, 50–52].

Routine treatment of clinical metritis with intrauterine antimicrobial agents (oxytetracycline; ampicillin; and cloxacillin), antiseptic chemicals (iodine solutions: 2% Lugol's iodine immediately after calving and again 6 h later as a preventive measure), systemic antibiotics (penicillin or one of its synthetic analogs; ceftiofur/third-generation cephalosporin/for 3–5 days; a single dose of ceftiofur s.c. in the base of the ear within 24 h after abnormal calving), intrauterine ozone treatment [53], supportive therapy (nonsteroidal anti-inflammatory drugs (NSAIDs)) such as flunixin meglumine, fluid therapy in case of dehydration, therapy with calcium and energy supplements in case of depressed appetite, and/or hormone therapy (oxytocin, $PGF_{2\alpha}$, or its synthetic analogs) are very variable [52]. According to our present knowledge, since intrauterine antibiotics and antiseptics may irritate the endometrium, it is not recommended. Routine use of prostaglandins is also controversial and requires further confirmation. Presently, systemic antibiotics (ceftiofur) and supportive therapy can be recommended for dairy farms [54, 55].

Cows with clinical endometritis having a palpable CL, treated by intrauterine infusion of cephapirin or $PGF_{2\alpha}$, had no significant difference in time to pregnancy [56]. However, higher pregnancy rates were detected in the treated groups than in untreated cows. Several reports have suggested that PGF2α treatment for clinical endometritis is at least as effective as any other alternative therapies with Lugol, polyvinylpyrrolidone-iodine, meta-cresol sulfuric acid, Lotagen, dextrose [57], or N-acetylcysteine combined with amoxicillin and clavulanic [58] and presents a minimal risk of harm to the uterus or presence of residues in milk or meat [52]. The treatment efficacy of clinical endometritis without an active corpus luteum, solely with prostaglandin, is limited; however, according to Lewis [59], such a treatment may be advantageous by stimulating the self-defense mechanism. The presence of bacteria in the uterus can be accurately diagnosed using an on-farm bacteriological culture system (Tri-plate). This way, we can contribute to our antibiotic use to be as rational as possible on our dairy farm [60].

Treatment of subclinical endometritis with antibiotics and/or PGF_{2a} or nonsteroidal anti-inflammatory drugs (NSAIDs) has been tried. However, controversial

results were achieved; further examinations are required [51, 61]. Intrauterine lavage with 500–600 ml of sterile physiological saline (35–40°C) on Day 30 after calving or intrauterine infusion with 50 ml of boiling sterile water (~100°C; "Samia-treat; SAT") of repeat breeding cows may improve pregnancy rate; however, they require further large-scale confirmations [62, 63].

4. Early diagnosis of postparturient metabolic disease in dairy cow

Ruminal fluid is one of the essential sources of energy metabolism in dairy cattle because they can ferment volatile fatty acids (acetate, propionate, and butyrate), which may complete some 60–70% of the energy requirements. Dairy cattle are usually in a negative energy balance (NEBAL) in the initial weeks of lactation. The energy intake during this period is less than half the energy requirements for milk production. Therefore, dairy cattle, through increased nonesterified fatty acid (NEFA) production, try to meet the gap between energy input and output during early lactation. The suppressed feed intake results in a lack of gluconeogenesis, which causes a lack of glucose for the complete oxidation of NEFA. The incomplete oxidation of fatty acids contributes to the increased production of ketone bodies (β-hydroxybutyrate/BHB/, acetone, and acetoacetate), which may cause ketosis and fatty liver [64, 65]. It is essential to mention that primiparous cows are more susceptible to metabolic stress during the transition period (3 weeks before to 3 weeks after calving) than multiparous cows [66]. According to Iwersen et al. [67], the electronic handheld BHBA measuring system using whole blood is a more valuable and practical tool for diagnosing subclinical ketosis than the commonly used chemical dipsticks, for example, Ketostix or Ketorolac. Szelényi et al. [68] reported that BHB concentrations measured at the farm by a portable handheld device (Precision Xtra, Abbot Laboratories) showed a significant correlation ($r > 0.92$, $P < 0.001$) with the results of samples evaluated in the laboratory before and after freezing.

More recently, metabolic health disorders (e.g., subclinical ketosis [69], subclinical hypocalcemia [70]) can be predicted with high accuracy during the transition period by using different wearable wireless biosensors such as ear-tag, halter (noseband), neck collar, or leg-tag-type sensors by measuring eating, ruminating, lying, and/or standing time reviewed recently by Cocco et al. [44] and Lee and Seo [48]. Paudyal et al. [71] suggested using "two indices that could identify different health disorders satisfactorily using animal level and pen level comparisons. The cow level index compared daily rumination with the 7-day rolling average of the same cow, and the pen level index compared daily rumination time with the average of the cows in the same herd. This approach utilized deviations in rumination, which accounts for variations in rumination time between the cows and daily variation within the same cow." Cows can be treated before developing clinical diseases, and in this way, costs associated with prolonged treatment and reduced milk yield can be decreased. The importance of early treatment of different metabolic disorders can be emphasized by the fact that dairy cows, after calving, may sacrifice their immune function to maintain lactation [69]. Therefore, they are also more sensitive to different infectious diseases such as metritis and/or mastitis [44, 45, 72].

Acute or subacute ruminal acidosis may develop due to decreased salivation during calving due to reduced period and intensity of chewing, especially when the ratio of concentrate is not limited to the days surrounding calving. Rumen acidosis may also negatively affect rumen motility and appetite. It can be diagnosed by measuring the pH value of the rumen fluid collected by a stomach tube or by

rumenocentesis in the field. However, the accuracy of diagnosing subacute ruminal acidosis is limited. Long-term measurement of reticuloruminal pH value using an indwelling and wireless data transmitting unit enables the evaluation of dietary composition. It allows for adjustments in feeding management in the field [73]. However, the currently available commercial bolus sensor systems with a pH sensor have an operational lifetime of no more than a few months; therefore, its general use in daily practice is presently limited [48].

As mentioned previously, a negative energy balance (NEBAL) can develop several days before calving and usually reaches its most negative level (nadir) about 2–3 weeks later and is used to be extended 10–12 weeks until the beginning of the usual breeding period [74]. A spontaneously NEBAL in dairy cows can represent a physiological state of undernutrition. The severity and duration of NEBAL are primarily related to differences in dry matter intake and its rate of increase during early lactation.

In the absence of precious livestock biosensors, it is essential to evaluate the body condition score (**Table 1**) using the 5-point condition scoring system (scale 0–5, in 0.25-point increments) to control nutritional management on the farm [75, 76]. Calving in moderate condition (3–3.5) and maintaining feed intake during the periparturient transition period are critical factors in reducing NEBAL and avoiding metabolic and reproductive disorders that are harmful to performance. Different levels of body condition score changes (ΔBCS) on fertility, milk yield, and survival of Holstein-Frieasian cows diagnosed with reproductive disorders (dystocia, twins, retained fetal membranes, metritis, and clinical endometritis), and other health disorders (subclinical ketosis, left displaced abomasum, lameness, clinical mastitis, and respiratory disease) between Days 5 ± 3 and Day 40 ± 3 after calving were examined in an extensive dataset involving almost 12 thousands dairy cows. It turned out that there were no significant interactions between body condition score changes and different health-related events. At the same time, excessive loss of BCS and reproductive diseases decreased reproductive performance and survival compared with other ΔBCS categories and health groups. It is essential to mention that excessive loss of BCS during early postpartum was characterized as having a higher milk yield [77].

Since body condition score is a strong predictor of subcutaneous fat reserves but, to a lesser degree, of skeletal muscle reserves, in periparturient dairy cows, a more precise evaluation of those reserves can be reached by separate ultrasonic examinations [78]. According to Schröder and Staufenbiel [79], backfat measurements can be done by placing a linear array transducer "lightly on the sacral area, vertically on an imaginary line connecting the pin (tuber ischii) and the hook (tuber coxa), at the point corresponding to the cranial end of the first coccygeal vertebra. The backfat measurements always include the skin thickness, and the profound fascia can be used as a landmark to distinguish backfat from the gluteal muscle." According to van der Drift et al. [80], "longissimus dorsi muscle thickness measurements can be done by placing linear-array transducer perpendicularly to the vertebral column on the transverse process of the fourth lumbar vertebra, at the site of the larger diameter of the muscle between the fasciae corresponding to the lateral edge of the multifidus dorsi muscle." The examination sites must be brushed to remove debris but not clip, and ultrasound gel must be applied to couple the probe surface with the skin [78]. Quantifying dairy cow body morphological traits by automatically processing images taken in a 3-D single-camera vision system makes it possible to predict body weight in dairy cows automatically. However, this model is unsuitable for monitoring short-term body weight variation or detecting anomalies in a cow's health status [81]. According to a recent review, while current research shows promising results in dairy cattle, there are still many

Body region	BCS												
	2.00	**2.25**	**2.50**	**2.75**	**3.00**	**3.25**	**3.50**	**3.75**	**4.00**	**4.25**	**4.50**	**4.75**	**5.00**
Thurl	"V" in appearance					"U" in appearance					flat		rounded
Ileal tuberosity	Angular				Rounded							Just visible	Not visible
Ischial tuberosity	Angular		Fat pad palpable	Rounded							Not visible		
Transverse processes of lumbar vertebrae	>0.5 visible	0.25 to 0.50 visible				0.10 to 0.25 visible		Only tips visible		Tips not visible			
Coccygeal ligament	Visible						Just visible	Not visible					
Sacral ligament	Visible							Just visible	Not visible				

Table 1.
The decision chart for body condition score (BCS) suggested by Ferguson et al. [75].

avenues to be explored for better automation of the whole-body weight estimation process [82].

Following parturition, regardless of NEBAL, a wave of follicular development begins 5–7 days after calving due to elevated plasma follicle-stimulating hormone (FSH). Three types of follicular development (**Table 2**) have been described and can be diagnosed in the field using ultrasonography [83]. The re-establishing pulsatile LH secretion can induce ovulation of a dominant follicle during early lactation [84]. Conversely, the developing NEBAL in early postpartum may suppress pulsatile luteinizing hormone (LH) secretions and reduce ovarian responsiveness to LH stimulation, thereby deterring ovulation. Non-ovulatory dominant or cystic follicles may prolong the interval for the first ovulation to 40–50 days after calving [84, 85]. It is important to mention that prolonged anovulatory anestrus may occur in 11–38% of dairy herds and can be associated with reduced fertility caused by NEBAL [86]. NEBAL can influence the timing of first postpartum ovulation, which negatively affects fertility [84, 87]. If a cow remains anovulatory for >50 days of lactation, it will be less likely to become pregnant during lactation and will be culled [88].

Plasma progesterone (P4) concentrations generally rise during the first two or three postpartum ovulatory cycles [89, 90]. NEBAL may reduce or moderate the rate of increase in P4 [89, 90]. Meanwhile, the metabolic clearance of P4 in high-yielding dairy cows can be increased by high energy and protein intake. As P4 plays an essential role in conceptus development and growth, a slower increase in P4 after ovulation may decrease embryo growth by Day 16 and may cause early embryonic mortality [91, 92].

Early postpartum NEBAL may adversely impact the quality of oocytes during the first 80–100 days after calving, which exerts another carryover effect on fertility [93, 94]. However, it is not easy to reconcile the impact of NEBAL on follicles and oocytes with the impact of high dietary energy on oocyte quality and the development of blastocysts in dairy cows [95, 96]. Extremes in BCS may negatively influence fertility [84].

Metabolic, endocrine, and postpartum health statutes may influence together fertility in dairy cows. Energy imbalance seems to be one of the most important factors, though we should consider the complex interactions of the factors mentioned earlier to improve fertility in our dairy farm [84]. Similarly, BCS, glucose, NEFA, or Insulin-like growth factor 1 (IGF-I) concentration from calving to AI cannot explain the low fertility rate [97, 98]. In contrast, Saby-Chaban et al. [99] have found a significant correlation between the prevalence of biochemical ketosis (BHB >0.15 mmol/l) measured by in-line in milk and fertility.

To prevent metabolic disorders in the periparturient period, such as milk fever, ketosis, fat cow syndrome, or rumen acidosis is essential to provide challenge fed during the dry-off period and early lactation. These diseases can increase the prevalence of reproductive disorders and reduce reproductive performance. Therefore, prevention is preferable to treatment and requires close attention to nutrition and management. Treatment of different metabolic diseases (hypocalcemia and ketosis) has been reviewed recently by Oetzel [100] and Mann et al. [101]. In addition, maintaining good body condition at calving and providing a

First dominant follicle (FDF)	FDF will ovulate 16–20 days after calving
	A turnover followes non-ovulation, and a new follicular wave will start
	FDF fails to ovulate and becomes cystic

Table 2.
Three types of follicular developments can be found immediately after calving [83].

high-density energy diet that does not produce a fatty liver in early lactation are also essential in minimizing the detrimental effects of NEBAL on the return of the estrous cycle after calving.

5. Accurate detection of estrus in dairy cow

Estrous detection rate may contribute to low fertility results because of the low detection rate [102]. Van Vliet and Van Eerdenburg [103] reported that cow factors might also be contributing to low detection rates. Due to the relatively small size of the average dairy herds in several European countries (<50 cows) and the year-round calving pattern, the chances of having more than one cow in estrus simultaneously are somewhat limited. In this way, they cannot stimulate the intensity and length of each other estruses [103]. Another point of concern is the short duration of estrus. A previous study [103] showed that a substantial number of animals (40%) showed estrous signs for less than 12 h. The mean duration of estrus was 13.7 h in their study, in which they observed the cows every 2 h for 30 min. Others found that the high-yielding dairy cows (46.4 ± 0.4 kg milk/day) had a shorter duration of estrus (6.2 ± 0.5 h vs. 10.9 ± 0.7 h), fewer standing events (6.3 ± 0.4 vs. 8.8 ± 0.6), and shorter standing time (21.7 ± 1.9 s vs. 28.2 ± 1.9 s) than lower-producing dairy cows (33.5 ± 0.3 kg milk/day) measured at the same conditions [104].

The short duration of estrus on modern dairy farms emphasizes the importance of correctly determining the optimum time for artificial insemination [105]. Simple observation of the herd in the morning before and after milking, at midday, and late in the evening for 30 min is greatly recommended to determine estrus accurately under usual management circumstances. The use of traditional aids such as tailhead markings with chalk, paint, or crayon (the pin bones and the tailhead are painted), pressure-sensitive mount detectors using a colored fluid that fills a container when pressure is applied (these devices are fixed with adhesive to the hair over the midline just in front of the tailhead), camera-based recognition system for pressure-sensitive devices [106], estrous detection strip (applied to the sacrum) with a reflective strip which can be detected with an overhead camera [107], and/or detector animals (vasectomized or surgically altered bulls or androgenized nonlactating cull cows, heifers, or freemartin heifers with chin-ball marking harness) [108] may contribute to detect estrus accurately in the field. The recent development of a pressure-sensitive device is that when a certain threshold on mounts is reached, a light is activated on the device. Different flashing light patterns can determine whether a cow is in suspect heat, standing heat, or when it is ideal for AI [108]. Recently developed activity meters (activity behavior is classified as lying, standing, walking, active, or inactive/resting/) such as leg bracelets, neck collars, or ear tags [48], and/or electronic pressure-sensitive mount detectors [109] may improve the accuracy of estrous detection. The combined use of monitoring of estrous behavior and one or more estrous detection aids may enhance its efficiency. Similarly, combined use of biosensor data of animal activity with in-line monitoring of milk yield, milk flow rate, milk temperature, and electrical conductivity of milk [110, 111], in-line progesterone measurement [112, 113], and/or ruminating time and eating time (eating bouts) may increase the accuracy of estrous detection in the farm [48].

It is essential to emphasize that when standing heat is used as a predictor for ovulation (26.4 ± 5.2 h), only a limited number of cows display standing heat (58%), especially when few animals are in estrus at the same time. The onset of mounting behavior shown in 90% of estruses is the best predictor for ovulation

(30.0 ± 5.1 h); however, its limitation is that it cannot yet be assessed by estrous detection aids [114].

There are several other methods to detect estrus, for example, direct electronic sensing of the odors of estrous pheromones [115, 116], continuous measurement of vaginal temperature and conductivity [117], ventral tail base skin surface temperature [118], rumen reticular temperature [119], or external auditory canal temperature [120]; however, they need further developments before introducing them into the daily practice.

6. Determining the optimal time for artificial insemination

The mean duration of an estrous cycle in dairy cows is 21 days (between 18 and 26 days), and ovulation occurs 25–32 h after the onset of standing heat [108]. According to Roelofs et al. [114], the duration between the onset of estrus and ovulation is 29.3 ± 3.9 h, while the onset of the first standing estrus and the time of ovulation is 27.6 ± 5.4 h [121]. Since the chances for pregnancy after artificial insemination (AI) are much higher when ovulation occurs within the survival time of sperm [105], it is essential to inseminate the cow within 12 h after the onset of estrus, namely during the last half of standing heat; therefore, the a.m./p.m. rule was developed as a guide for AI. This guideline recommends that cows observed in estrus in the morning should be inseminated in the afternoon, and cows observed in estrus during the afternoon should be inseminated the following morning [105]. In contrast, if we inseminate the cows at a similar time each day or employ an inseminator service, there is no need to follow the a.m./p.m. rule if heat detection is accurate, the insemination technique is good, and semen fertility is high on the farm [122–124].

The optimal time for artificial insemination (AI) after the onset of increased activity measured by pedometers is between 5 and 17 h [114], while according to Maatje et al. [125] and Yoshioka et al. [126], it is between 6–17 h and 10–18 h, respectively. When neck-mounted collars were used to detect estrus, the highest pregnancy rates were reached for primiparous and multiparous cows when they were inseminated between 13–16 h and 9–12 h after the onset of estrus, respectively [127]. At the same time, this difference between primiparous and multiparous cows could not be confirmed by Roelofs et al. [114]. When pressure sensing devices were used to detect estrus, the optimal time for AI felt between 4–12 h [109] and 12–18 h [128] after the onset of estrus, respectively. In comparison, artificial insemination had proven to be the most effective when cows were inseminated at 12 h after the onset of estrus [129].

According to Van Eerdenburg et al. [130], cows (n = 100) were detected with a scoring system in estrus. Of these animals, 50% showed standing heat (58% reported by Roelofs et al. [131]), and only 64 of the 100 cows achieving a score of >50 were presented for insemination; 98% did indeed ovulate. The other 36 were <45-day postpartum and were not inseminated [132]. The milk yield, parity, follicular size, and ovulation time were not correlated with the estrous behavior score. The animals that ovulated 0–24 h after the first ultrasonographic examination scored more than twice the number of points (188 versus 65 points) as those that ovulated 24–48 h after the first scan (P = 0.045). If ovulation occurred >48 h after AI, only 15% of the cows became pregnant (**Figure** 2). Cows that did not show overt signs of estrus and thus scored <100 points in the scoring system had a high chance of ovulating after 24 h and should therefore be inseminated again or given GnRH (or agonist) at the time of insemination [132].

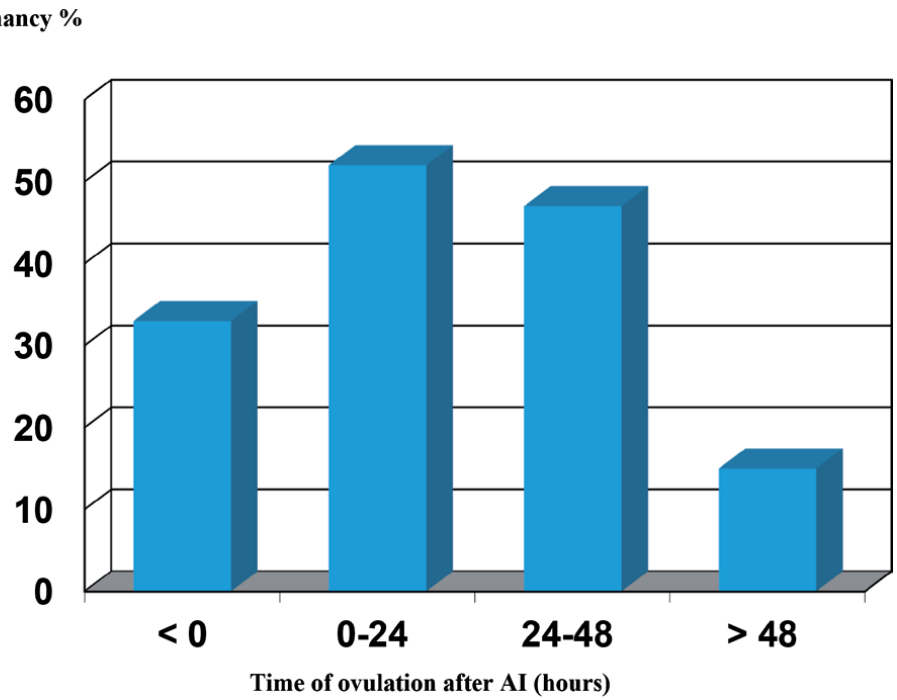

Figure 2.
Pregnancy rates at Day 28 concerning ovulation time after AI. Ovulation time <0 indicates that the cow had ovulated before the initial ultrasonographic examination adapted from [132].

Ultrasonography can also detect ovulation, since it is characterized by the abrupt disappearance of the large ovulatory follicle [132, 133]. The duration between the onset of the increased number of steps and ovulation can be 29.3 ± 3.9 h. In contrast, the period between the end of the increased number of steps and ovulation is 19.4 ± 4.4 h, measured by a pedometer. Pedometers may detect estrus accurately (83%) and appear to be a promising tool for predicting ovulation in dairy cows [131], while monitoring P4 alone is not sufficient to predict ovulation [134].

A progesterone (P4) assay of plasma or milk as an indication of true estrus clearly demonstrated that 7–22% of cows showing estrus had abnormal levels of P4 at the time of AI [135]. Bulman and Lamming [136] found that 15% of cows were inseminated during inappropriate stages of the follicular phase. However, a further 15% were inseminated during the luteal phase, while according to O'Connor [137] up to 15% of the cattle presented for insemination are really not in heat. When such cows are inseminated, they do not conceive, or it leads to abortion if they have been pregnant [135]. The number of artificial inseminations performed at the wrong time in the practice can be reduced by performing ultrasonographic examination [132, 133] or by using different diagnostic kits such as on-farm milk progesterone tests [138], in-line progesterone measurements [113, 139], or on-farm heat detection kits for detecting lactoferrin in cervical mucus [140] or by measuring the electrical resistance of vaginal fluid [137].

To eliminate the requirement for estrous detection and to optimize the timing of insemination relative to ovulation, different fixed timed artificial insemination (TAI) protocols were introduced into daily practice (**Table 3**). The TAI protocols may provide similar pregnancy rates per AI when compared with those of classical reproductive management systems, based on estrous detection and hormonal therapy when necessary. However, before selecting any protocol, it is always very important

Protocol names	Treatments	TAI	References
OvSynch	Day 0: GnRH, Day 7: PGF, Day 9: GnRH	**Day 10**	Pursley et al. [141]
Modified OvSynch-1	Day 0 a.m.: GnRH, Day 7 a.m.: PGF, Day 9 p.m. (30–36 h after PGF): GnRH	**Day 10 a.m.** (16–20 h after GnRH)	Pursley et al. [142]
Modified Ovsynch-2	Day 0 a.m.: GnRH, Day 7 a.m.: PGF, Day 8 a.m.: PGF, Day 9 p.m. (30–36 h after PGF): GnRH	**Day 10 a.m.** (13–16 h after GnRH)	Rheinberger et al. [143]
Shortened Ovsynch	Day 0: PGF, Day 2: GnRH	**Day 3** (16–20 h after GnRH)	Stevenson et al. [144]
Double-Ovsynch	Day 0 a.m.: GnRH, Day 7 a.m.: PGF, Day 10 a.m.: GnRH, Day 17 a.m.: GnRH, Day 24 a.m.: PGF, Day 26 p.m.: GnRH	**Day 27 a.m.**	Ribeiro et al. [145]
Cosynch	Day 0 a.m.: GnRH, Day 7 a.m.: PGF	**Day 9 p.m**. +GnRH	Geary et al. [146]
Presynch-14 – Ovsynch 10	Day 0: PGF, *Day 14: PGF, Day 24: GnRH,* Day 31: PGF, Day 33: GnRH	**Day 34**	Stevenson et al. [147]
Presynch-14 – Ovsynch-12	Day 0 p.m.: PGF, *Day 14 p.m.: PGF, Day 26 a.m.: GnRH*, Day 33 a.m.: PGF, Day 35 p.m.: GnRH	**Day 36 a.m.**	Martínez et al. [148]
Presynch-14 – Ovsynch 14	Day 0 p.m.: PGF, *Day 14: PGF, Day 28: GnRH,* Day 35 a.m.: PGF, Day 37 p.m.: GnRH (56 h after PGF)	**Day 38 a.m.** (16–20 h after GnRH)	Giordano et al. [149]
G-4-G – Ovsynch	Day 0: PGF, Day 2: GnRH, Day 6: GnRH, Day 13: PGF, Day 15: GnRH	**Day 16** (16 h after GnRH)	Bello et al. [150]
G-5-G – Ovsynch	Day 0: PGF, Day 2: GnRH, Day 7: GnRH, Day 14: PGF, Day 16: GnRH	**Day 17** (16 h after GnRH)	
G-6-G – Ovsynch	Day 0: PGF, Day 2: GnRH, Day 8: GnRH, Day 15: PGF, Day 17: GnRH	**Day 18** (16 h after GnRH)	
Modified G-6-G – Ovsynch	Day 0: PGF, Day 2: GnRH, Day 8: GnRH, Day 15: PGF, Day 17 p.m. (56 h after PGF): GnRH	**Day 18 a.m.** (16 h after GnRH)	Pursley and Martins [151]
PG-3-G – Ovsynch	Day 0: PGF, Day 3: GnRH, Day 10: GnRH, Day 17: PGF, Day 19: GnRH	**Day 20**	Stevenson et al. [147]
Ovsynch + PD: progesterone device (CIDR/PRID)	Day 0: GnRH + PD, Day 7: PGF – PD, Day 9: GnRH	**Day 10** (16–20 h after GnRH)	El-Zarkouny et al. [152]
Cosynch-5 + PD	Day 0: GnRH + PD, Day 5: PGF – PD, +6–8 h: PGF	**Day 8** +GnRH	Santos et al. [153]
Modified Cosynch-5 + PD	Day 0: PD, Day 5: PGF – PD	**Day 8** +GnRH	Colazo and Amdrose [154]

Table 3.
Fixed-timed artificial insemination (TAI) protocols.

to compare the results with the traditional methods used on the dairy farm. When estrous detection on the farm is good, $PGF_{2\alpha}$ treatment and AI at the observed estrus are recommended [155], while when estrous detection is poor, TAI protocols may be recommended. Recently published reviews [156–161] and meta-analyses [162–164] may contribute to selecting from an economic and management point of view the most suitable and most effective TAI protocol(s) for our dairy farm.

7. Accurate diagnosis of pregnancy and pregnancy loss in dairy cow

Pregnancy diagnosis plays an essential role in decreasing days open on dairy farms. Therefore, it is essential to select an accurate method for diagnosing early pregnancy and pregnancy loss (late embryonic and early fetal mortality) because the cost of each day open 100 days after calving may reach $4.00 [165] or €2.5–6.5 [166], respectively. Besides traditional pregnancy diagnosis (rectal palpation of the uterus or progesterone tests) [167–169], there are several new possibilities to diagnose early pregnancy on dairy farms. However, before introducing any new diagnostic test on our dairy farm, we must evaluate the accuracy of that particular test. Their results must be confirmed by the old diagnostic method to decrease the adverse effects of false-negative diagnoses. This can be caused by prostaglandin treatment to reduce the interval to the next AI service [167] or by using new resynchronization protocols in our dairy farm [170–172].

One of the most recent techniques for diagnosing early pregnancy on the dairy farm is B-mode ultrasonography. Under field conditions, ultrasonography may achieve accurate results from Days 25 to 30 after AI [173–175]. However, the accuracy of the transrectal ultrasonographic diagnoses greatly depends on the frequency of the transducer used, the surgeon's skill, the criterion used for a positive pregnancy diagnosis, and the position of the uterus in the pelvic inlet [176]. For example, if during ulrasonographic examinations performed between Days 24 and 38 after AI, we can find a uterus far cranial to the pelvic inlet compared with those cases when the uterus is located within or close to the pelvic inlet, we can make more incorrect nonpregnancy diagnoses [177].

Nonpregnant animals can be selected accurately by evaluating blood flow in the corpus luteum around Day 20 after AI, meaning we can substantially improve the reproductive efficiency of our herd [169].

Pregnancy protein RIA assays such as pregnancy-specific protein B/PSPB/, pregnancy-associated glycoprotein/PAG/, and PSP60, commercial ELISA, or rapid visual ELISA tests may provide an alternative method to ultrasonography for determining early pregnancy and pregnancy loss in dairy cows. However, the relatively long half-life after calving and pregnancy loss may limit the effectiveness of these laboratory methods for early pregnancy diagnosis in the field, especially when compared with a direct method such as transrectal ultrasonography [176]. Linear array/sector B-mode [178] and Doppler ultrasonography [169] may exceed the other diagnostic methods in the amount of information collected from each animal during scanning. However, their accuracies greatly depend on the operator's proficiency and availability [178].

A new technology (in-line milk analysis system) has already made the automatic collection of milk samples at milking robots or in the milking parlor to analyze progesterone to detect early pregnancy and pregnancy loss, respectively [113, 179, 180]. Bruinjé and Ambrose [113] reported high sensitivity (>95%) from Day 27 after AI, while the specificity was somewhat lower before Day 40 after AI. Any new biomarkers discovered for early pregnancy diagnosis may make it possible to diagnose

pregnancy loss much earlier, which may significantly contribute to increasing reproductive efficiency in our dairy herds. The importance of this technology would also be emphasized by its ability to identify pregnant and nonpregnant animals on time with no animal handling because even a simple transrectal examination of dairy cows can lead to increased plasma and salivary cortisol concentrations and changes in heart rate, heart rate variability, and behavior that are indicative of pain [181].

Although fertilization in the cow can be detected by measuring the early pregnancy factor with the rosette inhibition test, it is not a practical method; therefore, it needs further development. Recently found biomarkers such as interferon-tau-stimulated genes or microRNAs may help us diagnose early pregnancy in dairy cows; however, these tests need further development before their general use in the dairy practice [176].

8. Conclusion

Besides providing high-quality food for milk production and preventing metabolic disorders, it is essential to pay special attention to the first 100 days after calving. This is because the aim of our activity during the transition period is to prevent calving complications and uterine diseases and, if it is not possible, to treat them accordingly as soon as possible. Furthermore, it is essential to emphasize that if we would like to select a new diagnostic method or treatment protocol, it is always necessary to compare the results with the previously used test or treatment protocol.

Conflict of interest

The authors declare no conflict of interest.

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Section 5

Animal Welfare

Chapter 6

Effect of on- and off-Farm Factors on Animal Stress and Meat Quality Characteristics

Muawuz Ijaz, Mubarik Mahmood, Muhammad Kashif Yar, Muhammad Bakhsh and Sana Ullah

Abstract

Animal handling is a growing issue of concern in many countries around the world. Developed countries in particular show keen interest in the way animals are produced for processing. In such countries, animal welfare is increasingly becoming a primary matter in the process of keeping animals either as pets or for food and at homes or on farms. Not only are they protecting the rights of these animals but compromised handling of animal has negative effects on the carcass and overall meat quality characteristics. Poor quality animal and meat will have poor processing properties, functional quality, eating quality, and more likely to be unaccepted by consumers. Lesser attention has been paid by most developing countries on this issue. By this book chapter, it is expected that developing countries also take interest in proper on-farm and pre-slaughter handling of animals due to their beneficial effect on meat and carcass qualities.

Keywords: pre-slaughter handling, meat quality, DFD, PSE, meat industry

1. Introduction

Many physical and psychological stresses, the animals face before slaughtering. Physical stress includes high temperature, noise, less and disturbing space while psychological stress comprises social breakdown, pungent smell and new place [1]. Pre-slaughter stresses also come from mechanical injuries, starvation, lake of water and feed, loading and unloading of the animals which result in poor meat quality [2]. Apart from these stresses, meat quality is also affected from animal genetics, weather condition and environment [3].

Animal carcass and meat quality are linked with animal welfare. The welfare of an animal was defined by [4] as its condition as regards its efforts to cope with the environment. If an animal faces any type of difficulty and is not satisfied with its environment, the animal welfare is said to have been violated. At a farm level, environment, labor, any pathogen and other animals at the place have great effect on the carcass and meat quality. For example, if the food and environment of the animals is not clean and animals show some disease signs, it will directly affect their health and growth, resulting the less meat production and even the carcass can be wasted by meat inspector. Animal welfare is not merely defined by the good health, fitness and normal production, it is very difficult to measure the satisfaction of animal with its environment.

Warriss [1] stated the meat quality on functional and conformational basis. The required characters in a product are functional qualities and to fulfill the consumer need to that product is referred to as conformational qualities. Meat quality components include yield, gross appearance, wholesome, palatability and ethical concern [5]. The animal body which has been slaughtered for meat purpose is denoted by carcass. The poor quality of the carcass will definitely show bad meat appearance and quality. Smith and Grandin [6] reported that meat quality can be improved by proper handling and a good profit is achieved. In this chapter, pre-slaughter handling of animals and its effect on the carcass and meat quality will be studied.

2. Methodology

In many developing countries, animal welfare is compromised and a major concern on farms. Not only they compromised on the rights of these animals but poor handling of animal has negative effects on the carcass and meat quality attributes. Animal those are poorly handled either on farm or before slaughtering, their meat has poor processing properties, functional quality, eating quality, and more likely to be unaccepted by consumers. A little attention has been paid on such big issue of livestock. Consequently, keeping in view this scenario, the current manuscript has been developed to highlight the issue and spread the awareness by explaining effect of stress on carcass and meat quality. Therefore, it is expected that developing countries also take interest in proper on-farm and pre-slaughter handling of animals due to their beneficial effect on meat and carcass qualities.

3. Contributing factors to animal stress

3.1 On-farm factors

3.1.1 Use of growth promotors or hormone implant

Metabolic modifiers enhance the animal body condition and health. Dikeman [7] referred those metabolic modifiers are those substances which improve animal growth rate, feed efficiency, dressing percentage, shelf life, palatability, nutritional composition and meat quality and they are given to animals in the form of feed, injection or implants. Almost every country except EU, use these modifiers in meat producing animals. Their effects on meat quality of ruminants especially on beef cattle can be studied in [7–10] reported that steroid implants could increase the dark cutting frequency of meat. Although, there is low dark cutting of meat by 35% average in heifers and 69% in steers if we implant the growth promotors to 100 or more days [9].

3.1.2 Effect of animal gender

Heifers are more prone to dark cutting than steers [11–13]. Estrogen makes "fighting" or guarded nature of the animal [11, 12] found that heifers show more excitable nature than steers. Kenny and Tarrant [14] reported that high pH meat is produced in result of mounting behavior during the estrous period. Therefore, it is suggested to prevent estrus in meat heifers and these comprise spaying, progestins administration and GnRH immunization [15, 16]. Scanga et al. [9] reported that spayed animals had low occurrence of dark cutting. Bass et al. [17] reported that heifers do not show

much difference in glycogen level due to acute and short type of physiological reaction, while the steers have chronic stress. So, during regrouping and other activities, glycogen depletion is increased with higher pH_u and dark cutting.

3.1.3 Effect of animal age

The age of animal at slaughtering time has significance impact on meat quality along with animal breed, feeding plan, growth rate and live weight. As concerned with cattle, the meat color darkens with the age [18–23]. As the mature animal grows, myoglobin level increases which outcomes in dark color of meat [24–27]. Higher level of intramuscular myoglobin increases red color and reduces lightness of meat [28, 29]. On the other hand, the level of glycogen only increases the muscles oxidative capacity [30]. With the animal age, ultimate pH of muscle increases, thus darkening the color of meat.

3.1.4 Human-animal bonding

In the occurrence of dark cutting in ruminants, human behavior and gentle management play a key role. Lensink et al. [31] and Lensink et al. [32] reported that calves' meat showed a lighter color and lower pHu if they treated with positive human-animal bonding on farms than those treated with negative human-animal bonging. Lensink et al. [32] stated that gently handled animals were less nervous and showed more muscular glycogen as compared to those who treated roughly. Individual animal temperament and interaction with humans are noted and the difference is measured on farm and before slaughtering. Animals with high temperament are prone to stress. So, to minimize the stress and the risk of dark cutting, good animal handling practices should be applied.

3.2 Off-farm factors

3.2.1 Effect of climate condition and season

Dark cutting is prevented by the control of predisposing factors. The control environment is a difficult task. The cold or hot weather conditions before slaughtering deplete the muscle glycogen resulting in the increase of dark cutting [33]. Rainy cold season dissipates body heat of the animals [9] and it has stressful effects during the transport or at meat processing plants. Apart from wet or cold weather, warm season has great concern with dark color of meat [34–38]. Knee et al. [39] stated that during spring season, lambs show higher level of glycogen. On the other hand, [9] found the significance increase in dark cutting at the temperature of greater than 35°C due to low intake of feed during the hot weather. To minimize the stress of climate and season, trees, sheds or fences can be applied.

3.2.2 Marketing condition

The animals buying from markets have more occurrence of dark cutting than those receiving from farm to directly processing plants [40]. On the other hand, Warner et al. [41] reported no difference in dark cutting between those two groups, however, corticosteroid level in blood increased and muscle glycogen decreased with those treated saleyard management. Warren et al. [40] also verified the same case with animals coming though saleyard and directly from a farm. The traveling time, loading, unloading and water availability are other factors to be noted.

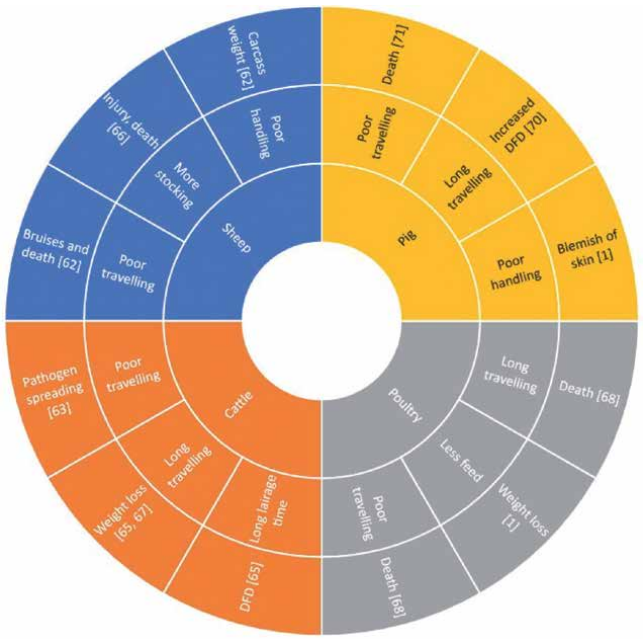

Figure 1.
Pre-slaughter animal handling and its effects on meat quality and carcass. The figure was adopted from the table of a review [51–58].

Apart from marketing system, dark cutting can be developed due to stressful activities before slaughtering of animals as these practices deplete muscle glycogen. To avoid fighting and other unknown damage, cattle are kept separately from new ones [42]. Mounier et al. [43] found an increase in dark cutting occurrence in those animals who were mixed with unfamiliar ones. Stress should be avoided before slaughtering and if stress happens accidently, it should be removed in a reasonable time.

3.2.3 Transport conditions

Due to improper handling, noise, weather conditions, loading, unloading, overcrowding and water and feed scarcity, transportation affects badly to meat and carcass quality [44, 45]. Tarrant et al. [46] reported that transporting stress can be minimized by some resting time on long traveling, adopting good handling techniques and loading ramps. Ferguson et al. [47] stated that short journey (less than 400 km) has no considerable effect on meat and carcass quality. Chulayo et al. [48] reported that as the transport distance crosses 400 km range, an additional stress affects the animal body leading to poor meat and carcass quality.

Warren et al. [40] said that there is higher occurrence of dark cutting in nervous cattle than those of calm ones. Schwartzkopf-Genswein et al. [49] advised that transporting vehicles should be divided into portions as in this way, small groups of animals are loaded, transported and unloaded. Grouping of animals with different sexes and from different farms should not be done [37, 40, 50]. The effect of pre-slaughter animal handling on meat and carcass quality is described in **Figure 1**.

4. On-farm factors affecting meat quality

It is necessary to get the data and history of concerned animal which helps in determining the meat quality. Poultry and pork (non-ruminants) are more

susceptible to meat quality defects as compared to beef and mutton (ruminants). Individual breed also varies to bear stress. For example, female, young and muscular breeds are more prone to stress as compared to other ones. Tarrant [46] said that transportation, morbidity and mortality have more effects on young calves. Layers are slaughtered at the later age of life, so their meat is tougher than broiler meat.

The growing and feeding system are great concern to meat quality as almost all animals are raised on farms. Argüello et al. [59] found that the kids grown on milk replacer had less water holding capacity and were lighter as compared to those grown by their dams. Sink and Caporaso [60] said that in flavor intensity of mutton, animal nutrition plays a key role and this flavor can be increased by the addition of legumes and grains. Vitamin E has a good role in raw and cooked muscles in terms of meat color, protein and lipid oxidative stability [61]. The fat is yellow if the animal is reared on grasses. If the animal is slaughtered after some medication or drug treatment, their residues can be found in the carcass. Such carcass is not suitable for processing as it will have low nutritional, hygienic and organoleptic quality.

5. Off-farm factors affecting meat quality

Animal farms are usually far away from main markets and meat processing plants. So, they are transported to other places with their loading and unloading and both should be carried out in a gentle way. Good loading and unloading techniques have been stated by [62]. Environmental stress is the main issue in transportation which have to face the animals such as high or low temperature, humidity, overcrowding and noise [63]. Meat quality is highly affected by over speed of vehicles, sudden breaks and long travel times. Appropriate measurements should be carried to minimize stress level.

Sometimes, animals are carried to markets where they face different type of stress in the form of hot or cold weather, noise, novel environment and new social grouping. Starvation and dehydration may be faced if feed and water is not given for a long time. Their skin can show different degrees of bruising. McNally and Warriss [64] stated that bruising prevalence varies from 2 to 8%. Adzitey and Nurul [62] addressed the possible measurements to reduce marketing stress and definitely pale soft exudate and dry firm dark. The pH values of meat for normal, PSE and DFD meat are shown in **Table 1**.

Feed and water should be offered to animal when they are transported and marketed. Council of Europe [68] said that proper feed and water should be offered to the animals at appropriate intervals during transportation. Without feeding and watering, they should not be left in this condition more than 24 hours of time. Although, if the destination is near and unloading will be done in sensible period,

Condition	Description	References
Normal	6.4 pH at 45 minutes	[65]
	5.5 final pH	[65]
PSE	Lower than 6 value of pH at 45 minutes	[66]
	5.3 final pH	[66]
DFD	6.4 pH at 45 minutes	[67]
	Final pH higher than 6.0	[43]

Table 1.
The range of pH values of normal, PSE and DFD meat.

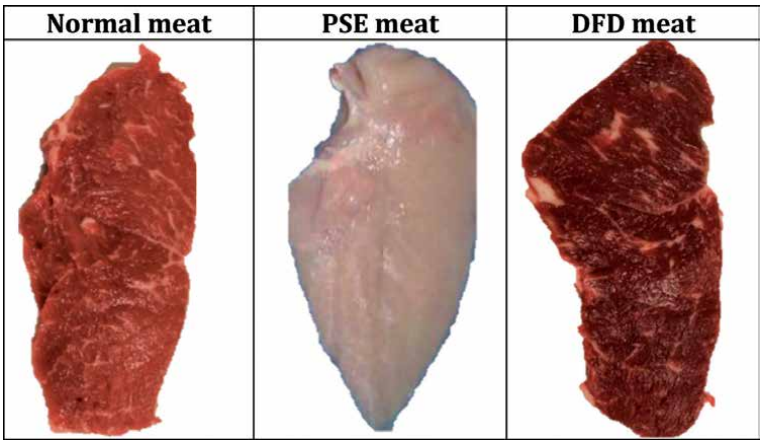

Figure 2.
The visual color of normal, PSE and DFD meat [the images were taken from PhD thesis of first author i.e., of Muawuz Ijaz].

this time can be increased. It is essential to provide them feed and water and if it is not fulfilled, depletion of muscle glycogen and weight loss can be occurred. Overfeed and water enhances gut fill, processing period and contamination and it should be avoided.

Before the slaughtering of animal, they are kept in the lairage which is a collection center of different animals. It is meant to recovery from stress and physical examination of the animal. If the animal is retained here for a longer time, some water and feed are also offered. Lairage is also a place to enhance meat quality of animals. On the other hand, it is a reservoir of pathogens as carcass contamination is increased by longer holding period [69]. Carcass and meat quality is damaged by electrical goads, beating and microbial contamination. So, the lairage measurements need to be improved according to animal desire.

Stunning is the last step of pre-slaughter handling of animals and it is done to make animal unconscious and insensitive. The equipment used for stunning has great concern with carcass and meat quality. Electrical stunning with high voltage can causes vertebral rupture, blood splash and low meat quality in pork [70]. Calkins et al. [71] stated that blood splash in pigs are due to electrical goads. To minimize stress level, well trained staff and stunning tool should be used.

Carcass and meat quality is affected by poor and inappropriate pre-slaughter handlings at the time of animal growing, loading, transferring, marketing, unloading, lairage and stunning [72]. Some of the concerned effects are; mortality of animals, low carcass yields, blood splash, broken bones, bruises, pathogen contamination and PSE and DFD. The color of normal, PSE and DFD meat is shown in **Figure 2**.

6. Conclusion

All the activities and procedures that animals go through before slaughtering are referred to as pre-slaught handling. When animals are transported and marketed from a farm to a slaughter house, these actions and processes are applied. To grow an animal to a specific age and weight, it requires much struggle and time and any abrupt change before slaughtering will definitely disturb the animal, affecting the carcass and meat quality, which leads to economic losses to the meat industry. Therefore, special focus should be emphasized during on-farm and pre-slaughter animal handling to ensure animal welfare and primal quality of meat.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 7

Social Dominance in South African Indigenous Zulu Rams

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Abstract

Social ranking is usually caused by limited access to resources such as feed, water as well as mating partners. In rams, social dominance is mostly related to physical traits such as body weight, horn size, body length and scrotal circumference. The objective of the study was to determine the relationship between physical traits of Zulu sheep rams and the establishment of social rankings. The social dominance rank was determined by a feed competition test using rams of the same age. Physical traits such as body weight, chest girth, horn length, scrotal circumference and withers height were measured for each ram. Sheep A was ranked first with a 100% number of wins ($P < 0.01$) followed by sheep E with an 86% number of wins ($P < 0.05$). A positive linear relationship between time spent on the feeder against the number of wins was not significant ($P > 0.05$). There was a significant positive correlation between the proportions of wins against horn length ($P < 0.05$) and chest girth length ($P < 0.05$). Time spent at the feeder was positively correlated with body weight and withers height ($P < 0.05$). Social dominance in Zulu sheep can be determined by particular physical traits such as horn length and chest girth.

Keywords: dominant, body weight and chest girth, feed, rank

1. Introduction

The importance of reproductive success in mammals, specifically in males, has caused the notion of social dominance to be investigated [1]. Social rankings are associated with inadequate access to various resources such as water, food, territory and shade [2]. Ungerfeld and González-Pensado [3] reported that mating performance is affected by hierarchical relationships in rams. Lower-ranking rams have restricted access to on-heat ewes [4]. Social rank is achieved by provoking other members in dominance fights [5].

Social rank is determined by physical traits such as body size, horn size and body condition score [3]. However, the authors further pointed out that rams with larger testicular circumference have a higher mating rate. Kabiraj et al. [6] reported that larger rams have larger testicular sizes. Larger and dominant rams have a tendency to suppress submissive rams to mate ewes [7].

Under an extensive farming system, where animals are reared in one herd, dominance ranking is likely to occur [2]. Research to understand the relationship between social behaviour and body developments in male ungulates is limited. In general, very little is understood about determinants of the individual rank of male

animals of any ungulates [2]. However, some studies were conducted on Merino and Border Leicester sheep [8], Bighorn sheep [9] and with three breed crosses, Wurttemberg, Ile De France, and Pirot Pramenka [2]. Such study has not been documented in indigenous Zulu sheep. Therefore, the objective of the study was to investigate the relationship between the physical traits of Zulu sheep rams and the establishment of the dominance hierarchy.

2. Materials and method

The experiment was conducted at the University of Zululand farm (South Africa), 28.8500° S, 31.8333° E in the small ruminant section. Eight rams of the same age (3 years) were used. To determine the social rank, the feed competition method by Maksimović et al. [2] was used. To initiate aggressive behaviour rams were subjected to fasting for 12 hours before data collection session. However, water was provided *ad libitum*. The feed was put in an immovable concrete container. Before the experiment commenced, body weight, pelvis length, horn size and chest girth were measured in all rams. To simplify observations and analysis, the animals were recorded by a CCTV camera giving a clear view as the animals entered the feeder site and during feeding [8]. After the experiment, the video was watched and analysed for evidence of dominant interactions. The position and time spent at the feeder by animals were also analysed. Furthermore, the activities of each ram were described using the method of Squires and Daws [8]. Each ram's activities were categorised as follows: (a) retained a fixed position at the feeder trough, (b) evacuated from the feed trough, (c) attempted re-entry on the feed trough and (d) on the edge. As ram entered the feeder, their identification number was noted and time spent in all four categories was recorded. Time spent by rams was recorded as they forced their way to the feeding trough, or attempted to enter the feeder, between adjacent aligned rams at the feeder. The ram trying to search for a space at the feeder was also noted. The number of contests between two rams was recorded, with a dominant and subordinate ram identified.

3. Statistical analysis

Data were analysed using SPSS. The number of contests and wins was recorded for each sheep. The number of wins was converted to a proportion and the binomial test was used to compare against the expected number of wins. A Pearson correlation analysis was used to test the relationship between body measurements, proportions of wins and minutes spent at the feeder.

4. Results

Tables 1 and **2** show the dominance-subordinate relationship in Zulu sheep rams. Ram A was ranked first with a 100% number of wins ($P < 0.01$) followed by Ram E with an 86% number of wins ($P < 0.05$). Ram D was ranked number 6 with only one win out of seven ($P < 0.01$). From the behavioural observations, rams divided themselves into two groups when they were introduced to the feed. The first group rapidly entered the feeder whereas the second group remained on the edge of the feeding trough or tried to find a space in the feed trough. Rams at the edge of the feeding trough struggled to push their heads in it by fighting rams, which were already feeding, but while trying to get to the feeding spot other rams pushed them

Subject	Opponent sheep								Number of contests	Number of wins	Proportion of wins	Rank on proportion of wins	p-value
Sheep	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**					
A		+	+	+	+	+	+	+	7	7	1.00	1	***0.0041
E	–	+	+	+		+	+	+	7	6	0.86	2	**0.0284
B	–		+	+	–	–	+	+	7	4	0.57	3	ns 0.3555
F	–	+	+	+	–		+	+	7	5	0.71	3	ns 0.1332
H	–	–	–	+	–	–	+		6	2	0.33	4	ns 0.2025
C	–	–		–	–	–	+	+	7	2	0.29	5	ns 0.1332
D	–	–	+		–	–		–	6	1	0.17	6	ns0.0630
G	–	–	–		–	–		–	6	0	0.00	7	***0.0072
Losses	0	3	5	5	1	2	6	5					

Reading across: + = dominant and – = subordinate. Reading down: + = subordinate and – = dominant. Blank spaces indicate that no contest was observed. NS = not significant.
**These results emanate from our own experiment.*
***P<0.05.*
****P<0.01.*

Table 1.
Dominance-subordinate relationship in Zulu sheep rams.*

	Time spent (%)			
Sheep Tag	**At the feeder**	**Displaced from the feeder**	**Attempting to re-enter feeder**	**Time spent on the edge of the feeder**
Ram A	98.7	0.7	0.6	—
Ram F	90.2	6.0	3.8	—
Ram E	85.9	10.2	3.9	—
Ram B	73.6	15.7	10.7	—
Ram H	60.0	13.3	26.7	—
Ram G	59.6	9.8	17.9	12.8
Ram C	50.3	15.0	34.7	—
Ram D	33.6	19.3	35.3	11.8

**These results emanate from our own experiment.*

Table 2.
Percentage of time spent at the feeder and in seeking re-entry.*

away. Throughout the feeding, there were contests and pushing between individuals. Due to the overcrowding at the feed trough, very few rams were able to occupy one spot without being pushed away. However, some rams were able to maintain their position at the feeder by positioning themselves laterally. In some instances, due to the need to feed and the pressure at the feeder, some rams (second group) gained access to the feeding trough by forcefully pushing their heads between two closely aligned individuals. Rams, which struggled to enter the feeding trough, were hindered by adjacent individuals, which were closely aligned to another ram, thus subsequently blocking entry to the feed trough. The behavioural sequences were complex at the feed trough, when one ram forced his way to the feeder; one or more rams lost their spot at the feeder. A dominant-ranking ram (i.e., Ram A) caused low-ranking rams to be submissive and flee. Submissive or subordinate rams were

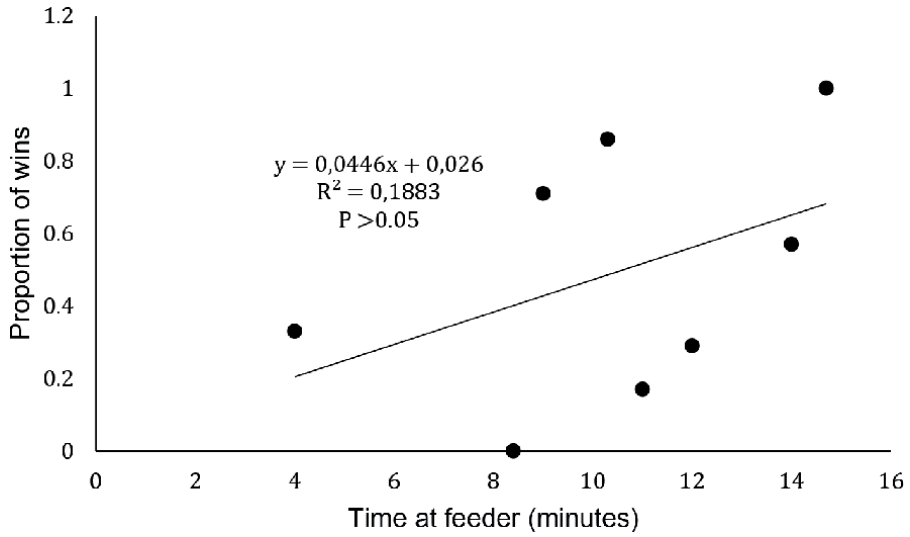

Figure 1.
Association between the proportion of wins and time at the feeder for each ram. *These results emanate from our own experiments.*

reluctant to try to find a feeding spot near the dominant ram due to the threatening behaviour displayed by the high- ranking ram. However, the submissive ram sometimes would wait for the dominant ram to stop its threatening behaviour and turn its head to feeding, and then a submissive ram would quickly move into the available feeding spot by doing so it would be allowed to stay and feed.

As shown in **Figure 1**, a relationship between time spent on the feeder against the number of wins was not significant ($P > 0.05$). **Table 3** exhibits a correlation between physical traits of Zulu sheep rams with proportions of wins and minutes at the feeder. There was a significant positive correlation between the proportions of

	Body weight (kg)	**Withers height (cm)**	**Scrotal circumference (cm)**	**Horn length (cm)**	**Chest girth (cm)**	**Time spent at the feeder**
Proportion of wins	0.165^{ns}	0.332^{ns}	0.147^{ns}	0.634^{**}	0.461^{**}	0.434^{ns}
Body weight (Kg)		0.572^{**}	0.513^{**}	0.160^{ns}	0.639^{**}	0.552^{**}
Withers height (cm)			0.115^{ns}	0.109^{ns}	0.361^{ns}	0.474^{**}
Scrotal circumference (cm)				0.696^{**}	0.099^{ns}	0.188^{ns}
Horn length (cm)					-0.247^{ns}	0.220^{ns}
Heart girth (cm)						0.060^{ns}

NS = not significant.
**These results emanate from our own experiments.*
***P < 0.05.*
****P < 0.01.*

Table 3.
Correlation between physical traits of Zulu sheep rams with proportions of wins and minutes at the feeder.*

wins against horn length ($P < 0.05$) and chest girth length ($P < 0.05$). Body weight was positively correlated with wither height ($P < 0.05$), Scrotal circumference ($P < 0.05$), chest girth ($P < 0.05$) and time spent at the feeder ($P < 0.05$). Withers height was positively correlated with time spent at the feeder ($P < 0.05$). There was a positive correlation between scrotal circumference and horn length.

5. Discussion

According to Keeling [10], in the absence of ewes, subordinate rams tend to initiate some of the agonistic interactions challenging high-ranking rams. After losing an encounter, the subordinate ram may display submissive behaviour to the winner. In the present study, agonistic interactions involved pushing, horn threat, head butt, chasing and low stretch. However, these behaviours were not quantified. These results were in accordance with the behaviours observed by Keeling [10] where low stretch and horn threat were observed as an agonistic behaviour in rams. The author described the low stretch behaviour as a threat display in which a ram lengthens its neck forward and horizontal to the ground. Pelletier and Festa-Bianchet [9] observed similar agonistic interactions during contests in rams. The observed behaviours included front kick, frontal clash, rubbing, butt, non-contact displacement, and horn threat. Similar to the present study, Roberts et al. [11] observed head butt behaviour as common in rams. This is when rams are slamming their heads together until one ram withdraws from the encounter. Squeezing was another behaviour, which was commonly observed in the present study where a ram would squeeze itself between closely aligned rams. This was similar to the observation by Erhard et al. [12] where a feeding ram would stand almost parallel to the wall holding the feed hopper and eventually other rams squeezed in between the feeding rams.

Roberts et al. [11] described 'win' as a situation where a sheep wins an encounter, either by initiating and displacing another sheep or fending off another ram trying to displace it. The 'loss' is a situation where one sheep loses an encounter, either by starting an encounter and failing to displace the other sheep or by being displaced by another individual initiating an encounter. The insignificant linear relationship between the proportion of wins and time spent at the feeder suggests that rams with a higher number of wins did not necessarily spend more time at the feeder. Some rams with a low proportion of wins were able to spend more time at the feeder compared to rams with higher proportions of wins. Low-ranking rams might have gained more access to the feeder by shifting laterally and squeezing themselves between aligned rams, or they would wait for the dominant ram to turn its head down and they would quickly rush into the available space. Squires and Daws [8] reported results similar observations. Dwyer [13] suggested that when the feeding space is limited there is an increase in displacement at the feeder, and some of the sheep will stop feeding and become non-feeders. Thus, a decrease in time spent at the feeder was due to forceful displacement and disturbances at the feeder of low-ranking rams by high-ranking rams. In dairy cattle, the reduction of feeding space per cow in dairy cattle increases agonistic encounters even if the feed is provided *ad libitum* [14]. The same was concluded in dairy goats by Jørgensen et al. [15], suggesting that there is a decrease in time spent at the feeder if there is limited feeding space.

A significant correlation between the proportion of wins against horn length and chest girth suggests that rams might have used their larger horns and wider girth to fight and gain feeding space in the feeder, thereby increasing the proportions of wins. Body mass and horn length mainly affect social rank [5]. Bergeron

et al. [16] stated that the heaviest males with long horns are generally at the top of the hierarchy. In this study, there was strong positive correlation existed between the proportion of wins and chest girth. Body weight was positively correlated with withers height, scrotal circumference, horn length, and time spent feeding but strongly and positively correlated with chest girth. In accordance with findings in this study, Maksimović et al. [2] also obtained a significant relationship between body weight and chest girth.

6. Conclusion

The social rank status of the ram can be determined by the proportion of wins. High proportions of wins seem to be related to high social rank status. Body weight was associated with chest girth whereas horn length was associated with scrotal circumference. The social rank of Zulu sheep is not affected by body size.

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Chapter 8

Laterally Coordinated Gaits in the Modern Horse (*Equus ferus caballus*)

Elise Renders and Alan Vincelette

Abstract

Besides "natural" gaits of walk, trot, and canter, selected horse breeds engage in the so-called artificial gaits, including the fox trot, running walk, and rack. Though some studies have been undertaken of these artificial gaits, the datasets are incomplete, sample sizes are small, and no comprehensive overview has been written. After reviewing the literature and detailing what is known about these artificial horse gaits, the authors present data of their own. Linear, temporal, and footprint parameters or given regarding artificial gaits of twenty horses total from specialized breeds. In addition to finding decreasing stride duration, lateral advanced placement, and tripedal support as one moves from the walk to the running walk to the rack, as with previous studies, we also found decreasing ipsilateral/diagonal step time ratios and increasing ipsilateral swing phase overlaps. Visually, the walk, trot, fox trot, and slow rack leave trackways of ipsilateral pairs in parallel rows, the running walk and canter leave trackways of isolated prints with the running walk pattern more symmetrical, and the fast rack, stepping pace, and pace leave trackways with an undulating pattern formed by diagonal pairs of hooves with hooves often crossing over the center line yielding a negative interior straddle.

Keywords: horse, gait, artificial, running walk, rack

1. Introduction

A gait is describable in terms of a footfall sequence of the landing and the lifting-off of feet as well as in terms of speed and coordination of limbs. When surefootedness is needed on slipper substrates walking gaits are best. In a walk, and at increasing speeds in a flat walk and running walk, as well as in the more laterally coordinated rack, the footfall sequence is RH-RF-LH-LF, and one foot is on the ground at all times allowing for great stability. At medium speeds a trot often occurs in horses, as it does in the zebra, wherein diagonal leg pairs move together in a two-beat rhythm, LH-RF and RH- RF. Such a gait seems to dominate in horse species that moved onto the steppes and plains where a softer substrate occurred along with a need for efficient locomotion over longer migration distances and speed to outrun predators. There is a moment of suspension in the trot and the trot is an easy travel gait for the horse and very efficient when a spring mechanism is present in the animal as occurred in monodactyl horse species. Some horse breeds now, and likely also in the past, could perform a medium-speed laterally coordinated gait of the

pace. In a pace ipsilateral leg pairs move together in a two-beat rhythm, LH-LF and RH-RF. There is again a moment of suspension. The pace is also found in camels and seemingly has some advantages in travel over certain substrates, such as sandy ones, and variegated terrains, as well as biomechanical advantages. The canter and gallop have a different rhythm and are asymmetrical. The footfall sequence starts off with RH-LH-RF-LF for the so-called left lead and LH-RH-LF-RF for the right lead canter or gallop and have moments where all four limbs are off the ground. The canter is slower than a running transverse gallop and the hind feet move in unison whereas in a running gallop the legs move more one after the other. The transverse canter and gallop are useful when speed is of the essence. In the rotary gallop, an advantage in sudden jumps or turns in the field, or needs instant acceleration, the hind front diagonal is replaced by the hind front lateral.

Horses will select their gait according to the stability, balance, maneuverability, efficiency, and speed needed within the framework of their anatomical configuration. They will also adjust their gaits to suit the substrate. Though we can forget this today, horses were animals of prey to which sudden outbursts and fast runs and maneuverability were a matter of life and death. Horses also have various traveling gaits when the herd is migrating. They also display unique ways of locomotion during courting and herding, or chasing away another horse they want out of the way for feeding reasons or to protect a foal (Renders, pers. observations). This is why they have a well-developed cerebellum responsible for determining the most suitable gait given a situation. Through continual study we are able to learn more and more about these gaits of horses. Here, in particular, we wanted to study horse gaits in their most spontaneous state, that is to say the gaits they most readily turn to and make use if left to their own devices. Still study of horse gaits does often necessitate, as here, observations on horses raised by trainers and so these gaits are in part "taught" to the horse, yet horses have an ability to switch between various gaits for diverse purposes, or to adjust the synchronicity and symmetry of the gait when desired. In other words though there has been a lot of focus on ideal gaits displayable in a show ring horses may often spontaneously opt for less ideal gaits that reflect more how they behaved in the past and in the wild.

2. Review of studies undertaken on "artificial" laterally coordinated horse gaits

Square ipsilateral horse gaits are those in which all four limbs operate relatively independently and involve a lateral footfall sequence of left hind (LH), left front (LF), right hind (RH), and right front (RF). The slowest and most basic square ipsilateral gait of the horse is the walk (see **Table 1**), an evenly-timed four-beat lateral-sequence and lateral-couplet gait, and so with a footfall sequence that can be demarcated with en dashes (–) indicating longer passages of time (LH–LF–RH–RF) and wherein ipsilateral limbs contact the ground closest together in time [1, 25, 26]. The walk typically occurs at speeds of 0.9 to 2.1 m/s, with a stride or cycle duration of 1.0 to 1.5 seconds, a stride frequency of 0.7 to 1.1 strides per second, and a stride or cycle length of 1.3 to 1.9 meters [2–5, 20, 22, 30–32]. On account of its slow speed and long stride duration there are no whole suspended phases where all four limbs are off the ground at once, or even contralateral suspended phases where both front limbs or both hind limbs are off the ground together, and so the hind limb makes contact with the ground for 55–75% of the stride or cycle length (i.e. a duty factor of 0.55 to 0.75), yielding support structures containing lots of three-feet support phases (54%), alternating with bilateral (24%) then diagonal (22%) support phases (see gait diagram a in **Figure 1**), i.e. 3-2D-3-2 L [3, 4, 22]. In the typical walk the

Gait	Walk	Running Walk	Rack or Tölt	Stepping Pace	Pace	Source (s)
Velocity (m/s)	0.9–2.1	2.7–4.5	2.7–7.6	3.8–7.4	8.0–12.3	[1–19]
Stride Duration (s)	1.5–1.0	0.75–0.68	0.66–0.42	0.62–0.54	0.54–0.34	[2, 3, 6, 8, 10–12, 16, 17, 20]
Stride Frequency (s^{-1})	0.7–1.1	1.3–1.5	1.4–2.4	1.6–1.9	1.9–2.9	[1, 3, 6, 11–14, 20, 21]
Stride Length (m)	1.3–1.9	2.0–2.2	1.7–3.2	2.0	3.5–6.3	[5–8, 11–13, 16, 17, 20, 22–24]
Duty Factor [Hind Stance %]	0.75–0.55	0.58–0.52	0.66–0.36	Unreported	0.44–0.27	[1–4, 7, 8, 10, 12, 20]
Front/ Hind Stance Ratio	1.04–0.95	0.92–0.86	0.95–0.85	0.98–0.80	1.02–0.93	[1, 3, 6, 11, 17]
Beats	4	4	4	4	2	[1, 25, 26]
Synchro-nicity	Even	Even	Even	Uneven	Even	[1, 25–27]
Lateral Advanced Placement	0.24–0.20	0.22–0.12	0.30–0.18	0.24–0.13	0.17–0.05	[1, 6–11, 13, 14, 28]
Lateral Advanced Liftoff	0.25	0.18–0.10	0.29–0.13	0.16	0.24–0.05	[7, 8, 18, 29]
Diagonal Advanced Placement	0.26	0.29–0.37	0.22–0.28	0.32	Not Reported	[9, 10]
Diagonal Advanced Liftoff	Not Reported	0.35–0.39	0.26–0.30	0.32	Not Reported	[9, 10]
Tripedal Support (% of stride)	54	23–5	37–0	25–15	0	[1, 4, 6, 7, 9–15]
Bilateral Support (% of stride)	24	50–67	22–46	48	63–93	[1, 4, 6, 7, 9–15]
Diagonal Support (% of stride)	22	27–16	48–3	27	0	[1, 4, 6, 7, 9–15]
Unipedal Support (% of stride)	0	0–8	8–65	Not Reported	0	[1, 4, 6, 7, 9–15]
4-Limb Suspension (% of stride)	0	0	0	0–10	7–37	[1, 4, 6, 7, 9–15]
Ipsilateral Stance Phase Overlap	0.44	Not Reported	0.63–0.64	Not Reported	0.61–0.68	[1, 4, 7, 13, 14, 28]
Overstep (% of stride length)	0.01–0.10	0.15–0.25	Not Reported	Not Reported	Not Reported	[5, 7, 8, 17, 22]

Table 1.
Temporal and linear kinematic parameters of the ipsilaterally coordinated gaits of horses reported in previous studies.

front foot duration of ground contact is 0.95–1.04 that of the hind foot, the timing of the steps are fairly even (lateral advanced placement of 0.20–0.24 of the stride length and diagonal advanced placement of 0.26), independent of each other or square (lateral advanced liftoff of 0.25 and ipsilateral overlap of front and hand limbs for 44% of the stride), and with each foot remaining flat on the ground as the one after it comes down [6, 7]. The hind feet often cap and so overlap the vacated location of the ipsilateral front feet when they land or slightly overstep them up to a

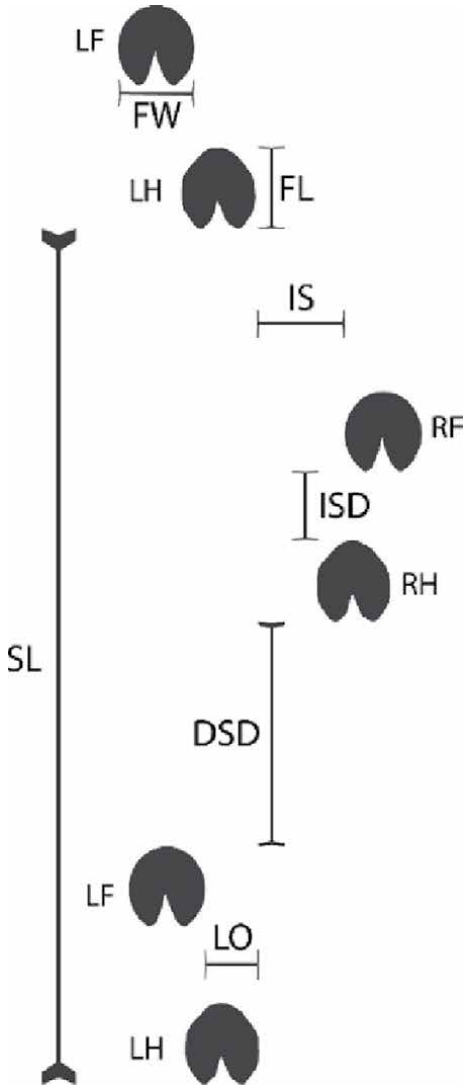

Figure 1.
Measurements taken of horse trackway (LH = left hind; LF = left front; RH = right hind; RF = right front). FW = footprint width; FL = footprint length; ISD = ipsilateral step distance; DSD = diagonal step distance; LO = lateral offset. IS = interior straddle; SL = stride length.

hoof-length, or more technically by 3–19 cm or 0.01 to 0.10 of the stride length, resulting in footprints in lateral pairs that are generally parallel with each other and just to the side of the centerline [5, 7, 8, 22, 32]. In gaited horse breeds the walk may be extended with the hind legs overstepping the ipsilateral front legs somewhat, in which case it is often called a flat walk or dog walk.

Most horse breeds, and the zebra, transition to the diagonal intermediate speed gait of the trot at around 2.0–2.5 m/s, but select horse breeds display other intermediate "artificial" square ipsilateral gaits. One such gait is the running walk (common to Tennessee Walking Horses, as well occurring at times in other horse breeds, such as the American Bashkir Curly Horse, Florida Cracker Horse, Kentucky Mountain Saddle Horse, Peruvian Paso Horse (paso llano), Smokey Valley Horse, Spotted Saddle Horse, and the Walkaloosa. Like the walk, the running walk is a fairly evenly timed four-beat lateral-sequence and lateral-couplet gait (see **Table 1**).

The running walk, however, occurs at a faster velocity than the regular walk and involves a lot of hind-leg extension and a bit more lateral coordination. Hence it can be demarcated with a hyphenated footfall sequence of LH-LF-RH-RF. The running walk typically occurs at a speed of 2.7–4.5 m/s, although it can be even faster in a few horses, with a stride duration of 0.68–0.75 seconds, a stride frequency of 1.3–1.5 strides per second, a stride length of 2.0 to 2.2 meters, and a hind-leg duty factor of 0.52–0.58 [1, 9, 10, 22]. At slower speeds the running walk is maintained via front legs pulling the horse forward while the hind longs engage in an extended stride with one hind foot lifting off while the other hind foot is still on the ground whereas the front feet employ shorter strides with one front foot coming down just as the other one is going up. Hence at slow speeds the running walk has a support structure with periods of tripedal, followed by diagonal then bilateral support, i.e. 3-2D-2L-3-2D-2L, though with much less three-limbed and more two-limbed support structures than in the standard walk, namely 15–23% tripedal, 50–67% bilateral, and 16–27% diagonal. At faster speeds the running walk can even have periods of single-foot support as both front feet are off the ground at the same time, though there is no period wherein both hind feet or all four limbs are off the ground. Thus at fast speeds the running walk has a support structure of 3-2D-1-2 L-3-2D-1-2 L, with a stride possessing 5–11% tripedal, 55–58% bilateral, 25–26% diagonal, and 2–8% single foot support structures [8–10]. At slow speeds the running walk has a lateral advanced placement of 0.22, a lateral advanced liftoff of 0.18, a diagonal advanced placement of 0.29, and a diagonal advanced liftoff of 0.35 the stride duration, while, at fasts speeds, these values reach 0.12, 0.10, 0.37, and 0.39, respectively, indicating more lateral coordination and less evenness to the gait [8–10]. Because the running walk involves a long hind-leg extension and the hind legs often scissoring out in an arc, two key kinematic features separate it from the standard walk. In the first place the front limbs are on the ground less than the hind limbs are, so there is a lower front to hind limb stance duration ratio of 0.86–0.92. Secondly, there is a very large overstep of hind feet over ipsilateral front feet of 15–50 cm, or around 15–25% of the stride length [8, 22]. Indeed typically the hind feet land near or just beyond the mid-point of the prior location of the front feet yielding footprint pathways of four insolated and almost evenly-spaced prints rather than prints with lateral or diagonal pairs. The running walk is a very smooth gait with little side-to-side or up and down movement of the horse's back, though it often involves the horses head nodding up and down with the movement of the hind limbs (Tennessee Walking Horse) or the forelimbs moving in a swinging arc (termino of the Peruvian Paso), and a slight rocking fore-to-back motion in the saddle.

Other gaited horse breeds transition from the slow walk to the intermediate "artificial" laterally coordinated gait of the rack or tölt. Such a racking gait is famous in the American Saddlebred, Icelandic Horse, Kentucky Mountain Saddle Horse, Mountain Pleasure Horse, North American Single-Footing Horse, Racking Horse, Rocky Mountain Horse, Smokey Valley Horse, Spotted Saddle Horse, and the Tennessee Walking Horse, but it also occurs in such breeds as the Aegidienberger, American Bashkir Curly Horse (curly rack with quick stride duration but lots of tripedal support), Garrano Horse (paso travado), McCurdy Plantation Horse (plantation gait), Mangalarga Marchador (marcha picada), Morgan Horse, Tiger Horse, as well as various Asian breeds [1, 10, 20, 33–36]. The rack or tölt is another fairly square and evenly-timed four-beat gait (see **Table 1**), with a footfall sequence of LH-LF-RH-RF, but one that involves more collection and quickness with a stride duration of 0.42–0.66, and a fair amount of lateral coordination, with the front limbs touching down close behind the hind limbs in time and remaining down until just after the opposite hind limbs contacts the ground, while the hind limbs of the

horse land close to but behind the contralateral front limbs in space, resulting in a bowed or wave-like footprint pattern, i.e. undulating, comprised of diagonal pairs [3, 4, 11–13]. At lower velocities of 2.7–3.8 m/s the rack is often labeled a mountain, pleasure, saddle, slow, stepping, style, or trail gait and possesses a 3-2D-3-2 L support structure–with 2–37% tripedal support, 25–46% bilateral support, and 16–48% diagonal support–a hind limb duty factor of 0.50–0.66, and a stride length of 1.7 to 2.2 m [11–13, 22, 36]. At such slow speeds the rack or tölt has a lateral advanced placement of 0.20–0.30, a lateral advanced liftoff of 0.27–0.29, a diagonal advanced placement of 0.23–0.28, and a diagonal advanced liftoff of 0.26, as well as a lateral overlap of 0.30 of the stride cycle, with the front feet having a stance duration 0.85–0.95 that of the hind feet [10, 13–16, 28]. At very slow speeds the rack often has ipsilateral feet forming footprint pairs on the ground or isolated prints resembling a running walk. At faster speeds of 3.9–7.6 m/s the rack or tölt is often called a performance, racing, road, show, or speed gait (or the contrarily labeled pleasure gait of the Rocky Mountain Horse), tends to become more animated with the front legs high-stepping or pitching out in an arcing motion, and bears 3-2D-1-2 L-3-2D-1-2 L or even 2D-1-2 L-1 support structures. A fast rack is hence often called a single-foot gait, especially in the North American Single Foot Horse and Racking Horse, and displays periods of single-leg support from 8 to 65% of the stride cycle depending whether there is contralateral suspension in the front alone (half rack) or both the front and hind feet (full rack), bilateral support of 22–40%, diagonal support of 3–14%, and lacking tripedal support structures. At such high speeds the rack or tölt possesses a lateral advanced placement of 0.18–0.30, a lateral advanced liftoff of 0.13–0.20, a diagonal advanced placement of 0.22–0.24, and a diagonal advanced liftoff of 0.30, as well as a hind limb duty factor of 0.36 to 0.49, fore feet on the ground only 0.62–0.87 the duration of the hind feet, and a stride length of 2.2–3.2 m [10, 13–17, 22, 28]. At these high speeds the rack lays down prints with diagonal pairs and consequently an undulating pattern of prints. The rack often exhibits some up-and down movement of the rear of the horse (seen in croup and tail), due to the horse pushing off forcefully from the hind feet, while the rider in the saddle can experience a slight side to side motion due to the increased lateral coordination of the limbs.

Many gaited horse breeds can also engage in a stepping pace, amble, or broken pace, i.e. an unevenly-timed shuffling 4-beat laterally coordinated gait, rather than an even 4-beat gait, so having a footfall pattern of LH-LF–RH-RF (see **Table 1**). A stepping pace, in particular, occurs in the Asturcón, Campolina (somber paso), Columbian Criollo Paso, Icelandic Horse (skeith tölt), Florida Cracker Horse (coon rack), Paso Fino (andadura), Racking Horse, Spotted Saddle Horse, and Tennessee Walking Horse. Such a gait has been little studied as it is usually not considered a desirable gait by breeders or horse breed associations, and, in fact, is often named depreciatively as a piggy-pace, slick pace, or pacey rack or pacey tölt, perhaps as it is somewhat uncomfortable for the rider since the horse's neck and back move from side to side. In the Peruvian Paso Horse, however, it is considered a desirable gait and named the sobreandando (agulillo; pasiamblado), and it is also prized in the Tiger Horse (glider gait or lateral Indian shuffle) and Virginia Highlander. In the stepping pace there is a large amount of lateral coordination of limbs as ipsilateral hind and front limbs lift off the ground just after each other and contact the ground just after each other and cause side-to-side motion for the rider. The stepping pace occurs around 3.8–7.4 m/s, with a stride duration of 0.54–0.62, a stride frequency of 1.6–1.9, a stride length of 2.0 m or more, and hind limbs remaining in the stance phase 0.80–0.98 as long as the hind legs. It possesses a lateral advanced placement of 13–24, lateral advanced liftoff of 10–16, diagonal advanced placement of 32–34, and diagonal advanced lift-off of 37–42. The stepping pace or sobreandando at slow speeds has a support structure of 15–25% tripedal support, 48% bilateral support, and 27%

diagonal support at slower speeds, or a 3-2 L-3-2D support structure, and at higher speeds there can be some single-foot support with alternating contralateral front and hind suspensions, i.e. 2 L-1-2 L-1, or even occasional suspension of all four legs from 1 to 10% of the stride duration with a 2 L-0-1-2 L-0-1 support structure [10, 15].

A related, but opposite, "artificial" gait, is the broken trot, which is usually called the fox trot and found in the Missouri Fox Trotter and the Marsh Tacky (swamp fox trot), or the marcha batida and found in Mangalarga Marchador horses (with occasional quadrupedal support phases). Though often described as a diagonal gait it is technically a lateral-sequence [LH–LF-RH–RF] diagonal-couplet gait, one in which the front limb lifts off just before the ipsilateral hind limb lands, giving the impression of the horse kicking its front legs forward with its hind legs [21, 27, 36]. As a broken trot, the fox trot also possesses an uneven four-beat cadence with some fore-to-back motion for the rider. It is also found in Icelandic Horses (brokk tölt), Paso Fino horses (paso corto and paso largo), Peruvian Paso horses (pasitrote), Columbian Criollo Paso horses (trocha), Spotted Saddle horses, and in the Nez Perce, Nokota Horse, the Tiger Horse (where it is called the glider gait or diagonal Indian shuffle), the Walkaloosa (Indian shuffle), and wild Mustangs. At high speeds it is sometimes called the fox rack or flying fox trot.

While most horse breeds switch from the trot to the gallop at around 4.0 m/s some horse breeds engage in the hard, straight, or true pace, the fastest lateral gait which involves heavily coordinated ipsilateral legs. The pace is a two-beat gait wherein ipsilateral legs lift-off and touch the ground simultaneously allowing for long stride lengths and great speed where it is called the flying pace or speed pace (see **Table 1**). The pace is found in Standardbred, Peruvian Paso (huachano), and Icelandic horses (flug skeith) and typically occurs at speeds of 8.0–12.3 m/s, and possess a stride duration of 0.34–0.54, a stride frequency of 1.9–2.9 strides/second, a lateral advanced placement of 0.07–0.17, a lateral advanced liftoff of 0.08–0.24, a front to hind limb stance duration ratio of 0.93–1.02, and a stride length of 3.5–4.8 meters [1, 4, 18, 19, 23, 24, 29, 37]. The pace has a hind-limb duty factor of 0.27–0.44 and a suspension phase of 7–37% of the stride wherein all four limbs are off the ground [16, 20]. The pace thus has a support structure of 2 L-0-2 L-0. Like the rack and stepping pace it forms footprints in the sand of diagonal couplets in an undulating pattern as the hind feet come down just behind the contralateral front feet. The lift-off and set down of the limbs can be somewhat asymmetrical (8%) between the right and left sides of the horse and the pace also produces considerable side-to-side rolling motion for the rider.

3. A study of laterally coordinated gaits in modern horses

In order to add to our knowledge of artificial laterally coordinated horse gaits the authors undertook a study of the linear, temporal, and footprint parameters of twenty horses. This study remeasured some of the parameters described in the investigations mentioned above, completed them by measuring missing variables such as the diagonal advanced lift-off, and finally introduced new parameters useful in elucidating different aspects of a gait, such as temporal evenness, linear symmetry, and gauge width (interior straddle).

3.1 Materials and methods

To elucidate the temporal and linear parameters of the various lateral gaits of living horse breeds, studies were undertaken on twenty horses comprising various gaited breeds, seven Tennessee Walking Horses, four Icelandic Horses, three

Peruvian Paso Horses, two Mangalarga Marchador Horses, two Rocky Mountain Horses, an American Saddlebred Horse, and a Spotted Saddle Horse (see **Table 2** below for additional details concerning the horses studied). The horses were ridden by professional trainers selected by the owners of breed-specific horse farms in the lateral gaits specific to the breed, including the walk, flat walk, running walk, rack, and stepping pace of the Tennessee Walking horse, the paso llano, sobreandando, and huachano of the Peruvian Paso horse, the tölt of the Icelandic horse, the marcha picada of the Mangalarga Marchador, the slow gait and rack of the American Saddlebred Horse, the running walk, rack, and fox trot of the Spotted Saddle Horse, and the show and pleasure racking gaits of the Rocky Mountain Horse.

We did not investigate other four-beat lateral gaits such as the classic paso fino of the Paso Fino horse which most often lays down tracks of understepping ipsilateral pairs, the lope or slow canter of Western horses which lays down footprints in contralateral pairs that angle the same way, or the mixed canter of the Tennessee Walker or Missouri Fox Trotter wherein the horse canters in front with contralaterally coordinated legs but walks in the rear (aubin, broken rocking chair canter, valhopp, wicky wack) or the reverse (traquenard).

The horses were videotaped in these lateral gaits at 60 frames per second (or 30 frames per second at the European horse farms where the speed was usually below 3.0 m/s) with advanced cellphone cameras located perpendicular to the plane of motion and five to ten meters back so that all of the horse's body was visible and foot contact with and lift-off from the ground could be observed. These videos were

Horse Number	Breed	Gender	Height (m)	Hoof Length (cm)	Hoof Width (cm)
1	Tennessee Walking Horse	Female	1.50	12.0	12.5
2	Peruvian Paso Horse	Male	1.51	12.0	11.5
3	Tennessee Walking Horse	Male	1.53	14.6	ca. 14.9
4	Tennessee Walking Horse	Male	1.63	17.8	ca. 17.1
5	Spotted Saddle Horse	Female	1.52	12.7	ca. 12.7
6	Peruvian Paso Horse	Male	1.33	11.4	ca. 11.9
7	Tennessee Walking Horse	Female	1.66	15.2	ca. 14.8
8	Icelandic Horse	Male	1.32	12.7	ca. 13.2
9	Icelandic Horse	Female	1.34	12.7	ca. 12.1
10	Rocky Mountain Horse	Male	1.53	15.2	ca. 15.2
11	Rocky Mountain Horse	Male	1.45	14.0	ca. 14.5
12	Tennessee Walking Horse	Male	1.54	12.7	ca. 13.4
13	Tennessee Walking Horse	Male	NA	NA	NA
14	Icelandic Horse	Female	1.48	NA	NA
15	Mangalarga Marchador	Male	1.50	NA	NA
16	Mangalarga Marchador	Male	1.48	NA	NA
17	Icelandic Horse	Female	ca. 1.27	NA	NA
18	Peruvian Paso Horse	Female	ca. 1.44	NA	NA
19	American Saddlebred	Male	1.55	16.5	ca. 16.1
20	Tennessee Walking Horse	Male	1.59	13.3	12.8

Table 2.
Information regarding modern horses studied (Original data).

analyzed frame-by-frame via Frame Player software to determine the timing of the lift-off and set-down of each of the horses' feet through a series of two stride cycles. From this recorded data various temporal parameters of the horses' gait could be determined such as stride duration and lateral advanced placement.

In addition, the footprint patterns left by eleven of these horses in the sand were photographed and measured in order to determine various linear parameters of the gaits such as stride (cycle) length and overstep amount. This was accomplished by raking smooth a 6x10 meter rectangular area in the sandy arena and using a tape measure or yardstick to measure various distances between the footprints after the horses performed gaits over it (see **Figure 1** for an illustration of measurements taken). Key physical parameters of each horse were also taken including height at the withers, and hoof length (see **Figure 2**).

The velocity of the horses was determined by noting how long it took on the videotape for the horse to complete a measured stride length as well as on occasion additionally timing the distance it took the horses to travel between cones or strips placed three to ten meters apart with a stopwatch. The first method was found to be the most accurate as it was difficult to accurately note when horses crossed various horizontal markers due to limitations of human perception as well as angular effects of distance of viewer from the markers. Hence the second method of using a stopwatch and delineated markers to measure speed was abandoned after the first six horses studied.

The temporal and linear parameters of the horse gaits were determined through videotape analysis or footprint measurements based upon the following definitions (modified from [38]):

Stride duration: Time in seconds taken to complete a stride cycle, i.e. time between successive left hind ground contacts. The stride duration tends to decrease in faster gaits.

Stride frequency: Number of strides per second, i.e. 1/stride duration.

Front stance phase: Percent of the stride duration the front limbs are on the ground.

Hind stance phase [here considered to be the duty factor]: Percent of the stride duration the hind limbs are on the ground.

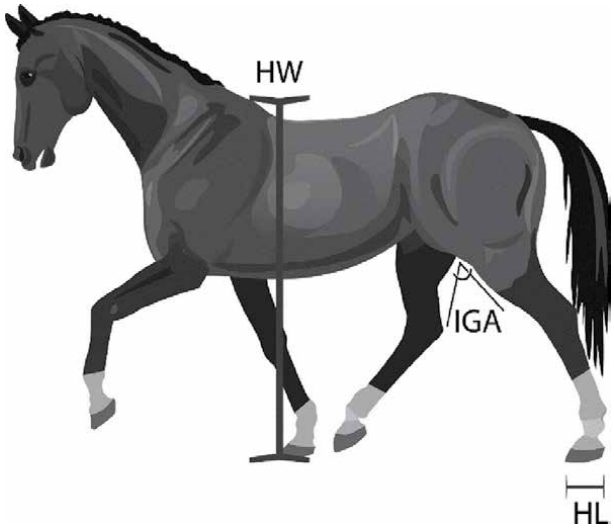

Figure 2.
Measurements taken of horse. HW = height at withers; HL = hoof length; IGA – Intergascular angle (maximum angle during gait).

Average stance phase: Average value of front and hind stance phase. A value over 0.50 indicates there is no suspension phase where all four-limbs are off the ground simultaneously.

Front stance/Hind stance ratio: Front stance phase value divided by hind stance phase value. A value near or over 1.0 tends to indicate a slower walking gait is occurring.

Time between ipsilateral steps: Time in seconds between the ground contact of ipsilateral hind and front hooves.

Time between diagonal steps (s): Time in seconds between the ground contact of contralateral front and hind hooves.

Ipsilateral/diagonal step time ratio: Average value of time between ipsilateral steps divided by time between diagonal steps. This ratio relates to evenness of the steps and hence is 0.75–1.25 in even gaits, and less than 0.50 or above 1.50 in asynchronous gaits. A value below 1.0 indicates lateral coordination of limbs and above 1.0 diagonal coordination of limbs.

Ipsilateral swing phase overlap: Percent of stride cycle ipsilateral limbs are off the ground together. This indicates degree of lateral coordination and exceeds 0.75 in highly laterally coordinated gaits.

Ipsilateral stance phase overlap: Percent of stride cycle ipsilateral limbs are on the ground together.

Overall ipsilateral overlap: Average value of ipsilateral swing phase overlap divided by ipsilateral stance phase overlap. Higher values indicate strong coordination between ipsilateral limbs wherein ipsilateral hooves lift off and set down close in time to each other.

Swing/stance phase overlap ratio: Average value of ipsilateral swing phase overlap divided by ipsilateral stance phase overlap. A lower value of 0.60 or lower indicates a slow gait with a long stance phase while a higher value indicates a faster gait with increased swing phase time and less stance phase time.

Lateral advanced placement [Limb phase]: Time between contact of ipsilateral hind and front limbs, expressed as percent of stride cycle and so divided by stride duration. This value is 0.20–0.25 in square gaits with limbs operating relatively independently, less than 0.20 in gaits with ipsilateral limb coordination and lateral couplets, and 0.30 or higher in diagonally coordinated gaits with diagonal couplets.

Lateral advanced lift-off: Time between lift-off of ipsilateral hind and front limbs, expressed as percent of stride cycle, so divided by stride duration. This value is below 0.20 in highly laterally coordinated gaits and above 0.30 in highly diagonally coordinated gaits.

Diagonal advanced placement: Time between contact of front limb and contralateral hind limb, expressed as percent of stride cycle, so divided by stride duration. A value below 0.20 indicates a high level of diagonal-coordination of limbs while a value above 0.30 indicates a high level of lateral-coordination of limbs.

Diagonal advanced lift-off: Time between lift-off of front limb and contralateral hind limb, expressed as percent of stride cycle or divided by stride duration. A value below 0.20 shows strong diagonal-coordination of limbs while a value above 0.30 indicates strong lateral-coordination of limbs.

Foot couplet time differential: Time in seconds between closest contact of hooves whether ipsilateral, diagonal, or contralateral front or contralateral hind.

Tripedal support phase: Percent of stride cycle three limbs are on the ground at the same time whether two front and one hind or two hind and one front. This value can be 50% or more of the gait in slower gaits and missing in faster gaits.

Bilateral support phase: Percent of stride cycle two ipsilateral limbs are on the ground at the same time whether left or right side. This value is less than 30% in diagonally coordinated gaits and above 50% in laterally coordinated gaits.

Diagonal support phase: Percent of stride cycle two diagonal limbs are on the ground at the same time, i.e. one front limb along with its contralateral hind limb. This value is less than 30% in highly laterally coordinated gaits and above 50% in a highly diagonally coordinated gait.

Unipedal support phase: Percent of stride cycle only one leg is on the ground, whether front or hind. Such unipedal support occurs in fast four-beat artificial gaits such as the running walk and rack or tölt.

Front contralateral suspension phase: Percent of stride cycle both front limbs are off the ground at the same time.

Hind contralateral suspension phase: Percent of stride cycle both hind limbs are off the ground at the same time.

Four-limb suspension phase: Percent of stride cycle all four limbs are off the ground together. This occurs in fast two-beat gaits, namely a trot or pace, and the canter and gallop.

Maximum rear intergascular angle: Inverted pendulum angle formed between contralateral gaskins (from stifle to hock) when both hind hooves are on the ground during gait (placement) or during protraction when both feet are in the air (suspension), or a combination of the two (see **Figure 2**). This value will be higher in gaits involving larger hind leg extension such as the running walk.

Stride length (cycle length): Distance in meters between the hoof top (toe) of successive left hind footprints. The stride length tends to increase as a gait gets faster.

Stride length/Horse height ratio: Dimensionless speed ratio found by dividing stride length by horse height at the withers. As gaits get faster this number increases. In walking gaits this value is usually less than 1.0, between 1.0–2.0 in intermediate speed gaits, while in very fast gaits it can be above 2.0.

Distance between diagonal steps: Measurement in centimeters between toe and heel of contralateral front and hind hoof prints.

Distance between ipsilateral steps: Measurement in centimeters between toe and heel of ipsilateral front and hind hoof prints.

Ipsilateral overstep/Stride length ratio: Distance between ipsilateral steps divided by the stride length. In the running walk with much hind leg extension as well as in fast laterally coordinated gaits such as the rack, stepping pace, and pace, the ipsilateral overstep can be 15–30% of the stride length.

Diagonal/Ipsilateral step distance ratio: Distance between diagonal steps divided by distance between ipsilateral steps. This value is below 0.50 in highly laterally coordinated gaits and above 1.0, often by a large margin, in highly diagonally coordinated gaits.

Average interior straddle [Gauge width]: Average distance in centimeters between quarters of contralateral front and hind hoof prints. In the walk and running walk this value is typically positive but in gaits with high-lateral coordination the hind limbs are free to come in or cross the centerline without interference and so this value is often negative.

Average foot pair lateral offset: Average distance in centimeters between quarters of closest hoof print pairs whether formed by ipsilateral, diagonal, or contralateral front or contralateral hind. This value is low when ipsilateral pairs of feet are close together but high when diagonal pairs are close together.

Foot pair lateral offset/hoof width: Ratio of average foot pair lateral offset divided by average width of hoof at quarters. This value is high for gaits with diagonal pairs landing close together in space but low for gaits with lateral pairs landing close together in space.

3.2 Results

Temporally the standard walk was characterized by a slow velocity (around 1.5 m/s), a stride duration over a second in length (1.16 seconds average), a front limb stance phase that is nearly as long as or even longer than the hind limb stance phase (1.02 front/hind limb stance phase ratio on average), a high ipsilateral/diagonal pair step time ratio (0.80 average), moderate ipsilateral swing phase overlap over the course of the stride (35.9% on average), and abundant tripedal support (52.9% of the stride duration) but not as much bilateral support (27.3%). The walk then is a relatively square and even four-beat gait with a lateral advanced placement of 21.6% and a lateral advanced lift-off of 24.1% of the stride cycle. The linear characterizations of the walk were not investigated here as previous studies have shown it to have a relatively short stride length (1.5–1.8 meters), with hind limbs that cap [forming direct register prints] or slightly overstep the front limbs (average intergascular angle of 33.3 degrees), and forming tracks in the sand characterized by lateral pairs in nearly parallel alignment if the horse is moving in a straight line [22] as can be seen in **Figure 3**. See **Table 3** and **Figure 4** below for additional temporal parameters and a gait diagram of the walk, as well as supplementary **Table S1**.

A few of the horse breeds exhibited the "artificial" lateral gait of the running walk, a faster variant of the walk emphasizing hind-limb extension (with an intergascular angle of around 59.0 degrees when both rear legs are on the ground), a pulling action with the front limbs, and often involving a counterbalancing mechanism such as nodding of the head and scissoring of the hind legs in the Tennessee Walking Horse, or an outward arcing path of the front limbs (termino) in paso llano of the Peruvian Paso Horse. The Spotted Saddle Horse, however, did not display much in the way of these counterbalancing mechanisms. The running walk is an even four-beat gait and is characterized temporally by a moderate stride duration (0.77 seconds on average, though a much quicker 0.58 seconds in the Peruvian Paso's paso llano), a high fore/hind stance duration ratio of 0.91 along with a high ipsilateral limb/diagonal limb step time ratio of 0.82, increased ipsilateral swing

Figure 3.
Footprint patterns of various laterally-coordinated gaits in modern horses. In the fast walk (a) there is a small stride length with a small overstep of ipsilateral hind feed resulting in distinct lateral pairs of prints in roughly parallel lines and a diagonal step distance much larger than the ipsilateral one. The fox trot and true fast trot (B) forms a trackway similar to that of the walk with lateral pairs of prints lining up more or less in parallel but possesses a greater stride length. In the running walk (C) there is a large overstep yielding no obvious pairs of prints as the ipsilateral step distance is nearly equivalent to the diagonal one but wherein the ipsilateral step distances and diagonal step distances are roughly equivalent with themselves. This should be contrasted with the gallop (D) which also lacks obvious print pairings but which has a much greater stride length and in which there is greater variance within the ipsilateral and diagonal step distances and a sequence of contralateral feet. In the rack of tölt (E) the ipsilateral step length is much greater than the diagonal one resulting in diagonal pairs of prints that form a bowed pattern with a large stride length and hind impressions that often cross over the centreline. In the stepping pace and true pace (F) there is an even greater stride length and the diagonal pairs of prints occur very close together as the ipsilateral step distance is much larger than the diagonal one. The scale is in centimeters.

Gait	Walk	Running walk	Slow rack or Tölt	Fast rack or Tölt	Stepping pace
Number of Horses Studied	3	5	3	7	7
Velocity (m/s)	ca. 1.53	2.86	2.70	4.23	3.74
Stride Duration (s)	1.16	0.77	0.62	0.53	0.61
Stride Length (m)	NA	2.30	1.68	2.16	2.12
Stride Length/ Height	NA	1.48	1.20	1.51	1.43
Hind Stance Phase (Duty Factor)	0.63	0.54	0.54	0.48	0.49
Front/Hind Stance Ratio	1.02	0.91	0.94	0.93	0.94
Ipsilateral/Diagonal Step Time	0.80	0.82	0.89	0.73	0.46
Foot Sequence	Lateral	Lateral	Lateral	Lateral	Lateral
Closest Foot Couplet Timings	Lateral	Lateral	Lateral	Lateral	Lateral
Beats	4	4	4	4	4
Synchronicity	Even	Even	Even	Even	Uneven
Limb Coordination	Independent	Front Alternating	Front Alternating	Front/ Hind Alternating	Ipsilateral
Intergascular Angle (degrees)	33.3	57.5	40.0	41.4	42.3
Ipsilateral Swing Phase Overlap	35.9	61.6	55.7	66.5	75.2
Lateral Advanced Placement	21.6	22.2	23.5	21.2	15.6
Lateral Advanced Liftoff	24.1	17.3	20.1	18.0	12.3
Diagonal Advanced Placement	28.3	27.7	26.5	28.8	34.4
Diagonal Advanced Liftoff	25.8	32.4	29.7	32.5	37.3
Tripedal Support (% of stride)	52.9	9.4	11.7	2.3	3.6
Bilateral Support (% of stride)	27.2	55.5	50.5	57.2	67.0
Diagonal Support (% of stride)	19.9	35.1	37.8	27.3	19.3
Unipedal Support (% of stride)	0.0	0.0	0.0	13.2	9.6
4-Limb Suspension (% of stride)	0.0	0.0	0.0	0.0	0.0
Ipsilateral Overstep (% of stride)	NA	18.8	8.7	28.3	29.0
Diagonal/Ipsilateral Step Distance	NA	0.94	2.99	0.37	0.24
Interior Straddle (cm)	NA	0.1	−0.6	−5.1	−1.1
Foot Pair Lateral Offset (cm)	NA	1.3	3.2	8.3	9.0
Foot Pair Offset/Hoof Width	NA	0.11	0.25	0.59	0.79
Footprint Pattern	Lateral Pairs	Equidistant	Lateral Pairs	Diagonal Pairs	Diagonal Pairs

Table 3.
Average temporal and linear kinematic parameters of ipsilaterally coordinated horse gaits (original data).

phase coordination (61.6% of stride), and much less tripedal support (averaging 9.4% overall) and more bilateral support (55.5%). The lateral advanced placement in the running walk averaged 22.2% and the lateral advanced liftoff 17.3% of the stride duration reflecting the greater degree of lateral coordination than in the standard walk. The velocity of the running walk averaged 2.9 m/s, with a stride length of 2.3 meters, an ipsilateral overstep of the hind over the forefoot that

Figure 4.
Gait diagrams of the walk, running walk, and paso llano of a horse.

averaged 18.8% the length of the stride. The trackway of the running walk consistently produced four footprints separated from each other and located fairly equidistant from each other (diagonal/ipsilateral step distance ratio of 0.94; see **Figure 3**). See **Table 3** and **Figure 4** for more of the temporal and linear parameters of the running walk and paso llano as well as gait diagrams, as well as supplementary **Table S2**.

The other common "artificial" lateral gait studied here was the rack found in the Tennessee Walking Horse and Rocky Mountain Horse and the similar gait of the tölt in the Icelandic Horse, which again is an even four-beat gait, though at a faster speed (averaging 3.8 m/s in the rack). In terms of temporal parameters, the rack or tölt is characterized by a relatively short stride duration (averaging 0.56 seconds), with a high ipsilateral/diagonal step time ratio of 0.79, a high forelimb over hindlimb stance duration ratio of 0.93, and a fair amount of lateral coordination (averaging 63.3% of the swing phase). The lateral advanced placement averaged 21.9% of the stride length and the lateral advanced lift-off 18.7%, while the hind limbs did not stretch forward as much at maximal protection as in the running walk (average intergascular angle of 41.0 degrees). Tripedal support was quite low (5.2% of stride cycle) while bilateral support was high (55.2%), and there was occasional

single foot support in some horses (around 13.2% of the stride cycle in fast racks). Perhaps the biggest difference, however, occurred in the prints themselves. At slower racking speeds, the ipsilateral feet form pairs with the hind foot overstepping the front foot (or on occasion form a trackway resembling a running walk with mostly isolated prints). In the fast rack, which had an average stride length of 2.2 meters, and an average ipsilateral overstep of 28.3% of the stride length, the footprint impressions are comprised of diagonal pairs with the hind foot understepping the front foot and forming a bowed or wave-like pattern in the sand (with a low diagonal/ipsilateral step distance ratio of 0.37). As observed in previous studies [22] there was very little distance separating the contralateral hoof impressions (see **Figure 3**), indeed when the rack is performed at fast speeds the hind foot understeps the front foot and due to lack of interference often crosses over the centerline and overlaps with its diagonal partner. This is reflected in a low interior straddle averaging −5.1 cm. **Table 3** and **Figure 5** display more of the temporal and linear parameters of the racking gait along with gait diagrams. See supplementary **Tables S3** and **S4**.

Some of the horses also engaged in the stepping pace characterized by an uneven four beat gait. Such a gait was very ipsilaterally coordinated with a lateral advanced placement averaging 15.6% and a lateral advanced lift-off 12.3%, along with an average ipsilateral swing phase overlap of 75.2%. Though the front/hind stance duration ratio was high (0.94), the ipsilateral/diagonal step time ratio was very low (0.46). Three-limb support was very also very low (3.6%), bilateral support very high (67.0%), and in some horses there was occasional single-foot support as well (for 9.6% of the stride length). The stepping pace took place at an average velocity of 3.7 m/s and had an average stride length of 2.1 m, ipsilateral overstep/stride length of 29.0%, an intergascular angle averaging 44.3%, and the footprint trackway in the sand occurred as close diagonal couplets as in the rack (0.24 diagonal/ipsilateral step distance ratio; see **Figure 3** and supplementary **Table S5**). **Table 3** and **Figure 6** show additional temporal and linear parameters of the stepping pace.

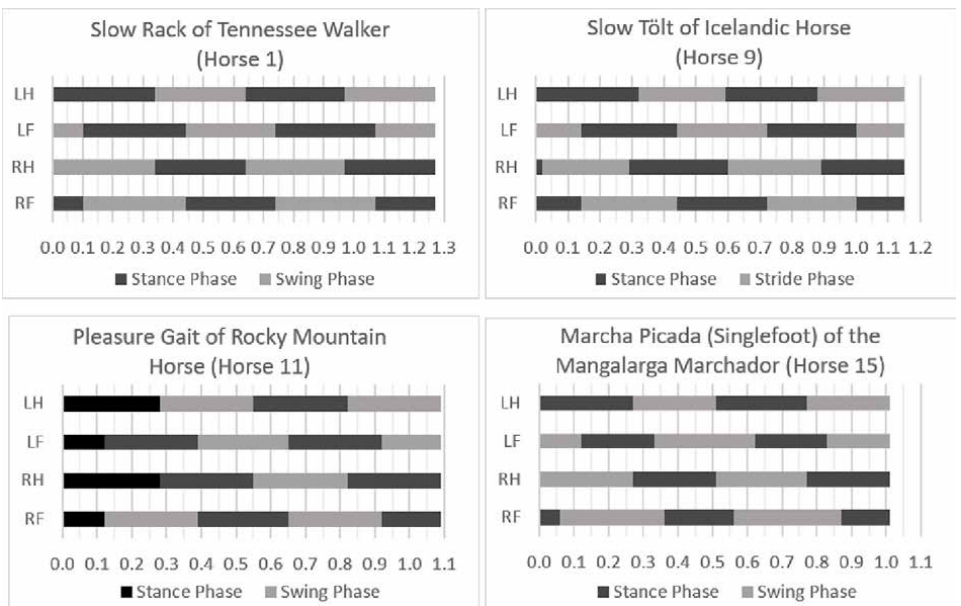

Figure 5.
Gait diagrams of the rack or tölt of a horse.

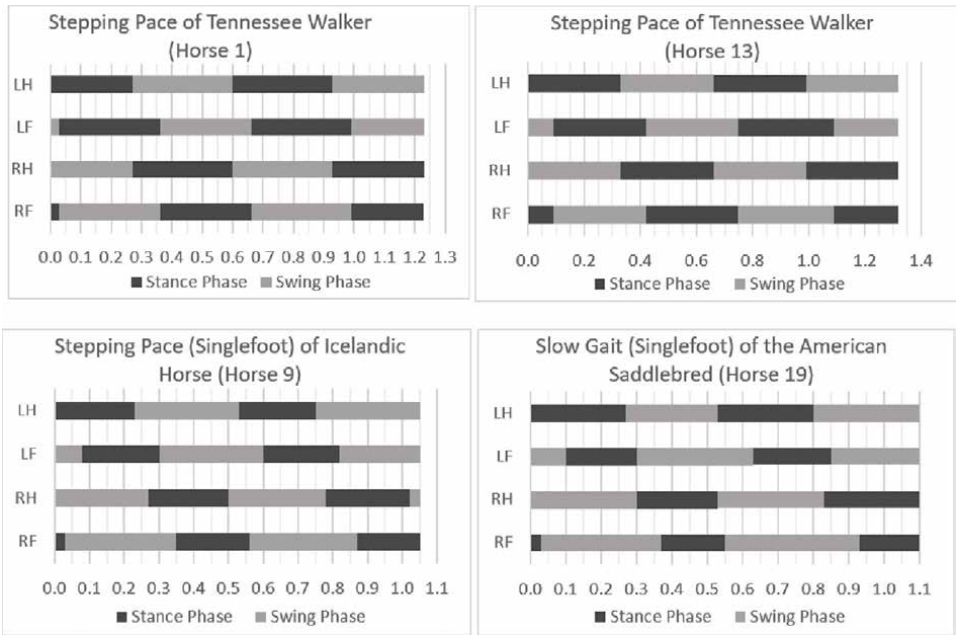

Figure 6.
Gait diagrams of the stepping pace of a horse.

Only two horses displayed a true or hard pace, i.e. an even two-beat gait with heavy ipsilateral coordination above 80% (with an average lateral advanced placement of 5–10% of the stride duration and 75–90% bilateral support through the stride cycle). This occurred at a relative slow speed for the pace, however, of around 3.4 m/s and so there were no suspension phases nor single-foot support structures (see **Figure 7** and supplementary **Table S6**).

Finally, three of the horses could also perform the fox trot gait, which is a lateral-sequence but diagonal-couplet running gait occurring at around 3.3 m/s. Hence the fox trot is usually considered a diagonal gait. The fox trot has very unusual temporal parameters accordingly, and was characterized by a stride duration averaging 0.62, lateral advanced placement of 38.4% and lateral advanced lift-off of 31.7%. The forelimb/hindlimb stance duration ratio was low (average of 0.86) while ipsilateral/diagonal step time ratio was super large at 4.11 due to a delayed front step on each side. There was in addition a large amount of diagonal support (62.8% of the stride

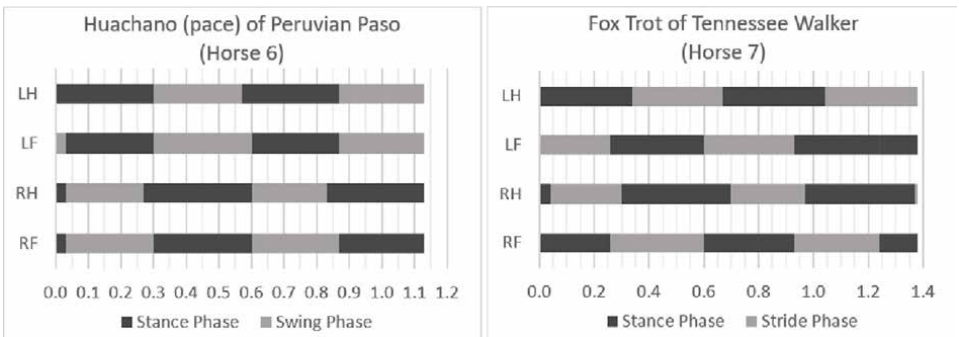

Figure 7.
Gait diagrams of the pace and fox trot of a horse.

cycle) and occasional single-leg support (2.8%), along with minimal ipsilateral swing overlap (27.1% of the cycle). See **Figure 7** for a diagram of some of the fox trots displayed by the horses and supplementary **Table S7**.

3.3 Discussion

Though definitely forming a graded spectrum, the various laterally coordinated gaits of horses can be distinguished kinematically and in terms of their footprint patterns (see **Figure 3**). The standard walk has a stride duration over a second in length, as well as a front/rear hind stance phase ratio around or over 1.0, and a stride length around 1.6 meters with a velocity of 1–2 m/s (see supplementary **Table S1**). The legs operate independently of each other (ipsilateral swing phase less than 50% and advanced lateral placement and liftoff around 20–25% of the stride length) and is very even (ipsilateral/diagonal step time ratio of 0.75 or higher). The intergascular angle, being 30 degrees or so, is small. The walk typically lays down of ipsilateral pairs of prints capping or sightly overstepping each other in two parallel tracks.

The running walk is faster than the walk at around 2.9 m/s with a stride duration of moderate size at 0.77 seconds and a lower front/hind stance phase ratio of around 0.91, and a longer stride length around 2.3 m (see supplementary **Table S2**). It has similar lateral advanced placement and ipsilateral/diagonal step time ratio as the walk, but is more laterally coordinated with the lateral advanced liftoff less than 20% and the ipsilateral swing phase overlap of 0.50 to 0.70. As it involves lots of rear leg extension it has a very high intergascular angle of 50–70 degrees. The running walk also has a diagonal/ipsilateral step distance ratio of 0.50 or more and so lays down a trackway of four independent hoof impressions nearly the same distance apart.

It should be noted that both the running walk and gallop lay down prints that are isolated from each other and so do not form pairs. However, the cycle length of the running walk trackway is shorter compared to the gallop trackway (2.2 vs. 3.1 meters), and while the step lengths of the running walk alternate between two short- and two medium-length steps which are fairly equivalent, in the gallop there is a short step (where contralateral feet spring off the ground), followed by a long step, followed by a medium-length step, in turn followed by another long step. As a result there is a noticeable asymmetry between the step lengths in the gallop, and the ratio of the stride length to horse height is around 1.8 or more versus the 1.4 or less found in the running walk [22].

The rack or tölt resembles the running walk in many ways but has a slightly higher speed of 2.7–4.2 m/s, a quicker stride duration around 0.62–0.53, and a smaller intergascular angle of around 40–41 degrees (see supplementary **Table S3**). At low speeds the trackway of the rack resembles the trot as it forms lateral pairs with the hind foot overstepping the front foot. At high speeds the rack forms diagonal pairs with the hind foot understepping the front foot and has a low diagonal/ipsilateral step distance ratio of 0.6 or less resulting in a bowed or wave-like pattern of prints. In the stepping pace the gait is heavily laterally coordinated with advanced lateral placements and lift-offs around 15.0% or less of the stride duration, an ipsilateral swing phase overlap of 0.70 or more, and an ipsilateral/diagonal step time ratio of 0.50 or less giving uneven beats (see supplementary **Table S6**). Even heavier lateral coordination results in a pace with advanced lateral placements and lift-offs of 12.0% or less, ipsilateral swing phase overlaps of 80% or higher, and an ipsilateral/diagonal step time ratio of 0.30 or less (see supplementary **Table S6**). The stepping pace and pace also lay down tracks in diagonal pairs forming an undulating pattern in the sand.

The fox trot is a diagonally coordinated but lateral-sequence gait and represents a sort of hybrid lateral-diagonal gait, due to it being an uneven and broken trot. In it diagonal couplets land close together in time with the front foot coming down just before the hind one, yet it yields ipsilateral pairs that are capped or wherein the hind foot slightly oversteps the front foot at high speeds. The fox trot occurred at around 3.0–3.7 m/s with a stride duration of 0.60–0.67. It is characterized by an extremely large ipsilateral/diagonal step time ratio of 2.0–6.0 and a lateral advancement placement of 30–40% of the stride length resulting a gait with a large amount of diagonal support (50–70%) and capped or slightly overstepping ipsilateral couplets (see supplementary **Table S7**).

Some of the key discriminatory parameters are the stride duration (sec) and front/hind stance duration ratio, which tend to above 1.0 in the walk but below 1.0 in the running walk, rack, and stepping pace; the ipsilateral/diagonal step time ratio, which tends to be above 0.75 in the square gaits of the walk, running walk, and rack, and below 0.5 in the strongly laterally coordinated gaits of the stepping pace and pace and 2.0 or higher in the fox trot; the ipsilateral swing phase overlap, which is usually below 0.40 in the walk and fox trot, 0.50–0.70 in the running walk and rack, and above 0.70 in the stepping pace and pace; lateral advanced placement and lateral advanced lift-off, which are around 0.15–0.25 in square gaits such as the walk, running walk and rack, 0.05–0.17 in heavily laterally coordinated gaits such as the stepping pace and pace, and above 0.30 in diagonally coordinated gaits such as the fox trot; diagonal advanced placement and diagonal-advanced lift-off, which are around 0.26–0.35 in walk, running walk, and rack, above 0.33 in laterally coordinated gaits such as the stepping pace and pace, and typically below 0.20 in diagonally coordinated gaits such as the fox trot and trot; the bilateral support phase which increases from 15 to 30% of the gait in the walk and fox trot to 40–60% in the running walk and 60% or more in the stepping pace and pace; the diagonal support phase which increases from 10 to 20% in the pace, stepping pace, and walk, to 20–40% in the running walk, and 50% or more in the

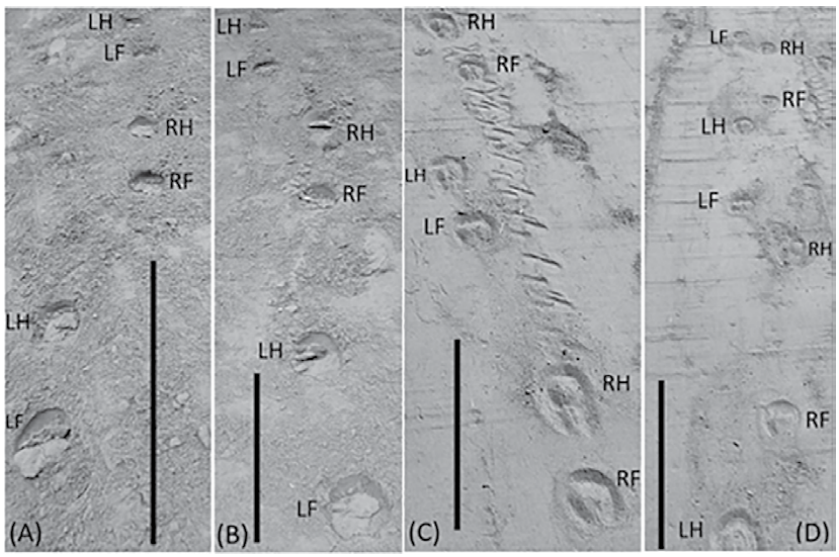

Figure 8.
Photographs of laterally-coordinated gait trackways of modern horses. A. Fast walk of a Tennessee walking horse (horse 12), stride length = 176 cm; B. running walk of a Tennessee walking horse (horse 12), stride length = 203 cm (horse 12); C. slow tölt with overstepping lateral pairs in an Icelandic horse (horse 9), stride length = 163 cm; D. fast tölt (stepping pace) with understepping diagonal pairs in an Icelandic horse (horse 9), stride length = 217 cm. Black bars are 50 cm long.

fox trot and trot; the maximum rear intergascular angle, which is around 30 degrees in a walk, 30–45 degrees in a rack and 45–70 degrees in the running walk; stride length which is around 1.5–1.8 m in the walk, 1.6–2.7 in the running walk and rack, and above 2.5 in the gallop; the diagonal/ipsilateral step distance ratio, which is typically below 0.5 in the fast rack, stepping pace, and pace, 0.5–1.5 in the walk and running walk; and finally foot pair lateral offset/hoof width which is usually less than 0.25 in the walk, fox trot, and trot, and 0.25 to 1.4 in the fast rack, stepping pace, and pace. Overall then our temporal and linear data matched that of previous studies.

Visually the walk, trot, and fox trot leave trackways with ipsilateral pairs in roughly parallel rows, the running walk and gallop leave trackways with isolated single hoof prints with the running walk pattern typically more symmetrical than the gallop pattern, and the fast rack, stepping pace, and pace leave trackways with an undulating pattern formed by alternating diagonal pairs of hoofs (see **Figures 7** and **8**).

Finally, it is worth noting that we observed an occasional asymmetry between the left and right side of the horse even in the so-called "symmetrical gaits" wherein, for example, the left front foot might lift off before the right front sets down but not the reverse.

4. Conclusion

Horses are famous for their variety of gaits. For this, and for their amicable character, and indeed ability to interpret human facial expressions and bodily movements, they have become valuable companions and helpers of humans. They were domesticated for riding and pulling carts and now have a large presence in sporting events such as dressage, jumping, and racing.

Most intriguingly besides the standard diagonal gait of the trot horses are capable of various laterally coordinated gaits such as the running walk and rack which allow for smooth riding for humans and efficiency of locomotion for the horse as well as offering stability and balance depending upon the substrate. Here we wanted to further examine such laterally coordinated gaits and look at what is known about them and expand upon our knowledge of the gaits of these magnificent creatures.

In this investigation we found temporal and linear parameters that can be utilized to discriminate the different laterally coordinated gaits of horses. It is true that these gaits often occur on a spectrum, such as the rack, stepping pace, and pace. However, one can select criteria such as percentage of ipsilateral coordination and footprint patterns to distinguish the gaits. For example, the running walk and racking gaits tend to have an ipsilateral swing phase overlap of 50–75% whereas the stepping pace has an ipsilateral swing phase overlap above 75%, while, at the same time, the running walk lays down isolated footprints, the slow rack lays down footprints consisting of lateral pairs, and the fast rack and stepping pace lay down footprints consisting of diagonal pairs. Though we did not stress it here there are also behavioral and physiological factors that can be used such as amount of head nod or croup displacement or foot sprinting. Further study on these areas would be warranted, as would whether or not a medium-speed rack lays down isolated footprints that resemble those of a running walk and the biomechanical differences between the pulling running walk gait and pushing rack gait. Further study would also be quite beneficial on these asymmetries found in gaits such as the rack, as well as study of the spontaneous gaits of horses when lacking a rider which seem to often weave from side to side rather than occurring

in a straight line (perhaps to help avoid a predatorial attack). It would also be good to measure the angle each of the feet make in relation to the centerline in each of the gaits. Finally we were only able to record video at a maximum of 60 fps, and used 30 fps on occasion for the slower gaits (rather than the more common standard of 120 fps), which gives a potential error of 0.017 seconds, though this can be reduced in half by taking account of how close to making ground contact the hooves are in a given frame. Indeed someday when technology improves capturing video of animals in motion at 480 or even 960 fps would be ideal for a potential error of less than a thousandth of a second.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

Other declarations

This study was approved by the Institutional Animal Care and Use Committee of St. John's Seminary, Approval # 20221.

Supplementary **Tables 2–3** and **Table S1–S7**, and **Figures 1–8** and **Figure S1** are original to this study and represent the data, art, or photography of Elise Renders and Alan Vincelette.

Videos are available upon request.

Appendices

Supplementary Tables S1-S7: Temporal and Linear Kinematics of Modern Horses in Various Gaits.

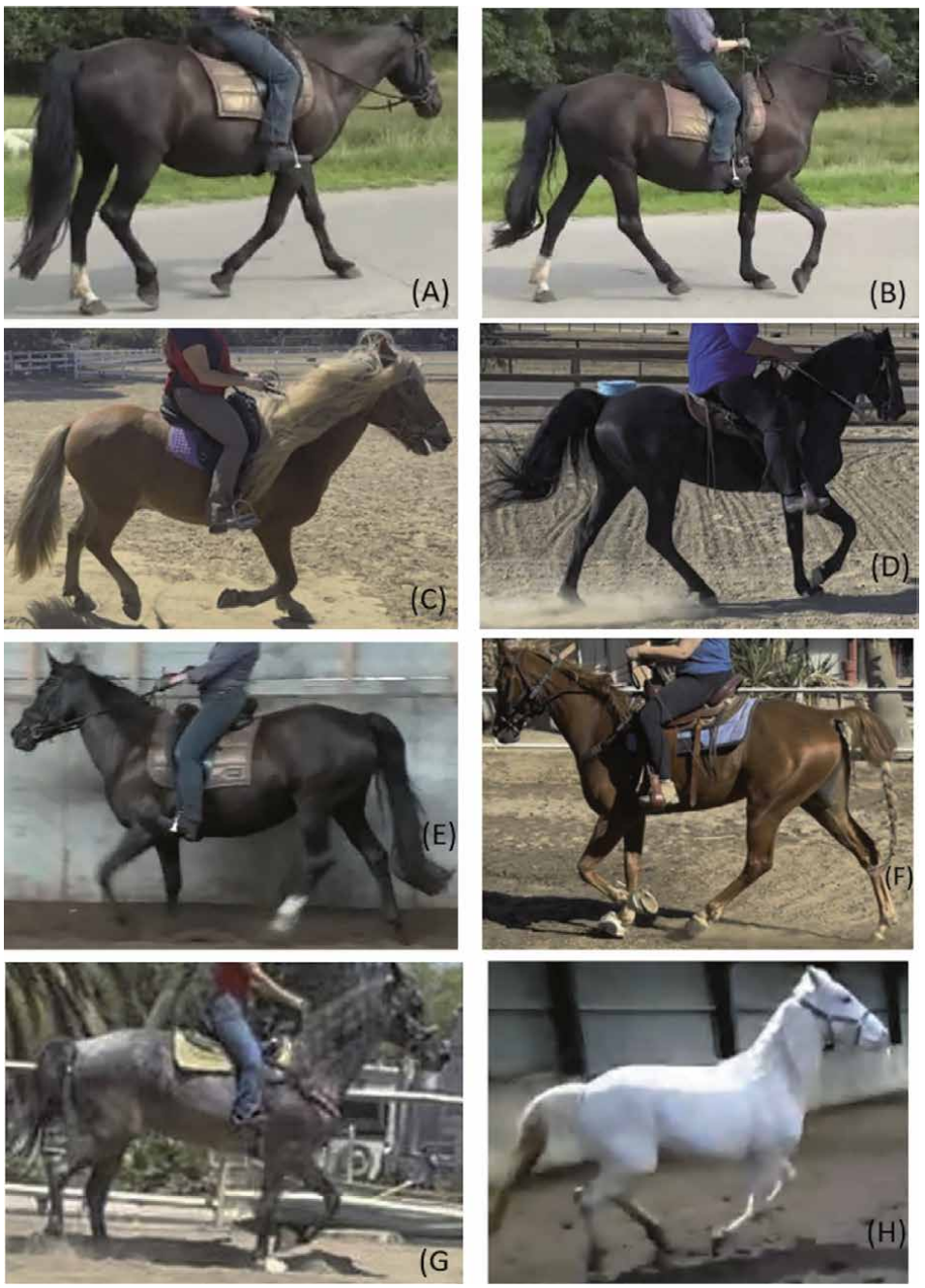

Figure S1.
Photographs of horses in various gaits. A. Tennessee Walker (Horse 1) in a slow walk; B. Tennessee Walker (Horse 1) in a stepping pace; C. Icelandic Horse (Horse 8) in a slow tölt; D. Rocky Mountain Horse (Horse 11) in a fast rack; E. Tennessee Walker (Horse 1) in a pace; F. Tennessee Walker (Horse 12) in a running walk; G. Tennessee Walker (Horse 7) in a fox trot; G. Tennessee Walker (Horse 20) in a gallop.

Traits	Horse number		
	1	**5**	**18**
Velocity (meters/second)	ca. 1.70	ca. 1.30	ca. 1.60
Stride Duration (seconds)	1.17	1.27	1.05
Stride Frequency (strides per second)	0.85	0.79	0.95
Front Stance Phase (% of stride)	62.4	65.6	63.8
Hind Stance Phase (% of stride)	61.1	62.6	64.3
Average Stance Phase (% of stride)	61.8	64.1	62.6
Front Stance/Hind Stance Ratio	1.02	1.05	0.99
Time Between Ipsilateral Steps (seconds)	0.26	0.27	0.25
Time Between Diagonal Steps (seconds)	0.33	0.37	0.28
Ipsilateral/Diagonal Step Time Ratio	0.77	0.73	0.89
Ipsilateral Swing Phase Overlap (% of stride)	39.0	36.1	32.6
Ipsilateral Stance Phase Overlap (% of stride)	64.3	66.1	63.4
Overall Ipsilateral Overlap (% of stride)	51.7	51.1	48.0
Ipsilateral Swing/Stance Phase Overlap Ratio	0.61	0.55	0.52
Lateral Advanced Placement (% of stride)	21.8	21.1	21.9
Lateral Advanced Liftoff (% of stride)	23.7	24.7	24.0
Diagonal Advanced Placement (% of stride)	28.2	30.4	26.4
Diagonal Advanced Liftoff (% of stride)	26.3	26.7	24.5
Tripedal Support (% of stride)	48.3	52.2	58.1
Bilateral Support (% of stride)	30.3	28.1	23.3
Diagonal Support (% of stride)	21.4	19.8	18.6
Unipedal Support (% of stride)	0.0	0.0	0.0
Front Contralateral Suspension (% of stride)	0.0	0.0	0.0
Hind Contralateral Suspension (% of stride)	0.0	0.0	0.0
Four-Limb Suspension (% of stride)	0.0	0.0	0.0
Intergascular Angle at Placement (degrees)	40.0	30.0	30.0
Stride Length (m)	NA	NA	NA
Stride Length/Horse Height (at withers)	NA	NA	NA
Distance Between Diagonal Steps (cm)	NA	NA	NA
Distance Between Ipsilateral Steps (cm)	NA	NA	NA
Ipsilateral Overstep/Stride Length Ratio	NA	NA	NA
Diagonal/Ipsilateral Step Distance Ratio	NA	NA	NA
Average Interior Straddle (cm)	NA	NA	NA
Average Foot Pair Lateral Offset (cm)	NA	NA	NA
Foot Pair Lateral Offset/Hoof Width Ratio	NA	NA	NA

Table S1.
Temporal Parameters of the Slow Walk (Original Data).

Traits	Horse Numbers						
	1	3	4	12	13	2	6
Velocity (meters/second)	2.35	2.95	3.39	3.10	ca. 2.50	3.42	2.93
Stride Duration (seconds)	0.72	0.83	0.77	0.79	0.75	0.57	0.58
Stride Frequency (strides per second)	1.39	1.20	1.30	1.27	1.33	1.75	1.72
Front Stance Phase (% of stride)	49.7	49.4	50.3	49.5	50.0	49.1	47.4
Hind Stance Phase (% of stride)	54.5	52.4	55.6	55.4	54.3	55.3	56.5
Average Stance Phase (% of stride)	52.1	51.8	53.0	52.4	52.2	52.2	51.9
Front Stance/Hind Stance Ratio	0.91	0.91	0.90	0.91	0.92	0.89	0.84
Time Between Ipsilateral Steps (s)	0.14	0.22	0.16	0.18	0.18	0.16	0.11
Time Between Diagonal Steps (s)	0.22	0.20	0.23	0.22	0.20	0.13	0.19
Ipsilateral/Diagonal Step Time Ratio	0.62	1.08	0.68	0.83	0.88	1.28	0.57
Ipsilateral Swing Phase Overlap (%)	70.1	52.3	65.0	62.1	58.5	49.5	72.7
Ipsilateral Stance Phase Overlap (%)	61.2	52.2	62.9	59.1	57.1	49.2	68.0
Overall Ipsilateral Overlap (% of stride)	62.9	52.2	64.0	60.6	57.8	49.4	70.4
Ipsilateral Swing/Stance Phase Overlap	1.15	1.00	1.03	1.05	1.03	1.01	1.07
Lateral Advanced Placement (%)	19.1	25.9	20.3	22.6	23.3	28.1	18.1
Lateral Advanced Liftoff (% of stride)	12.8	22.0	16.0	16.6	19.0	22.4	12.1
Diagonal Advanced Placement (%)	30.9	24.1	29.7	26.9	26.7	21.9	31.9
Diagonal Advanced Liftoff (% of stride)	35.4	28.3	34.3	32.8	31.0	27.2	38.4
Tripedal Support (% of stride)	9.7	7.8	8.5	12.1	8.7	11.4	12.1
Bilateral Support (% of stride)	61.8	48.2	59.5	54.8	53.3	43.9	63.8
Diagonal Support (% of stride)	28.5	44.0	32.0	33.1	38.0	44.7	24.1
Unipedal Support (% of stride)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Front Contralateral Suspension (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hind Contralateral Suspension (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Four-Limb Suspension (% of stride)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Intergascular Angle at Placement (°)	50.0	60.0	70.0	60.0	55.0	50.0	45.0
Stride Length (m)	1.69	2.45	2.61	2.45	NA	1.95	1.70
Stride Length/Horse Height (at withers)	1.13	1.60	1.60	1.59	NA	1.29	1.28
Distance Between Diagonal Steps (cm)	34.0	41.9	29.2	43.2	NA	39.5	31.8
Distance Between Ipsilateral Steps (cm)	23.5	45.7	54.6	51.4	NA	32.0	25.4
Ipsilateral Overstep/Stride Length Ratio	0.14	0.19	0.21	0.21	NA	0.16	0.15
Diagonal/Ipsilateral Step Distance Ratio	1.45	0.92	0.53	0.84	NA	1.23	1.25
Average Interior Straddle (cm)	2.4	−0.7	−0.7	−0.7	NA	4.0	−6.4
Average Foot Pair Lateral Offset (cm)	NA	NA	NA	NA	NA	1.3	NA
Lateral Offset/Hoof Width Ratio	NA	NA	NA	NA	NA	0.11	NA

Table S2.
Temporal and Linear Parameters of the Running Walk (Horses 1, 3–4, 12, 13) and Paso Llano (Horses 2, 6) (Original Data).

Traits	Horse Numbers				
	8	9	10	5	10
Velocity (m/s)	2.50	2.77	2.82	2.70	3.75
Stride Duration (s)	0.64	0.59	0.64	0.70	0.59
Stride Frequency (s^{-1})	1.56	1.69	1.56	1.43	1.69
Front Stance Phase (% of stride)	48.4	49.6	54.3	50.4	48.3
Hind Stance Phase (% of stride)	55.1	53.0	55.1	54.6	52.6
Average Stance Phase (% of stride)	51.8	51.3	54.7	52.5	50.4
Front Stance/Hind Stance Ratio	0.88	0.94	0.99	0.92	0.92
Time Between Ipsilateral Steps (s)	0.15	0.13	0.15	0.14	0.13
Time Between Diagonal Steps (s)	0.17	0.16	0.17	0.21	0.17
Ipsilateral/Diagonal Step Time Ratio	0.92	0.83	0.92	0.67	0.74
Ipsilateral Swing Phase Overlap (%)	58.9	58.2	50.1	66.2	65.2
Ipsilateral Stance Phase Overlap (%)	56.5	57.3	56.5	63.4	59.0
Overall Ipsilateral Overlap (%)	57.7	57.7	53.3	64.8	62.1
Swing/Stance Phase Overlap Ratio	1.04	1.02	0.89	1.05	1.11
Lateral Advanced Placement (%)	24.0	22.6	24.0	20.0	21.4
Lateral Advanced Liftoff (% of stride)	18.1	19.7	22.4	15.4	16.7
Diagonal Advanced Placement (%)	26.0	27.4	26.0	30.0	28.6
Diagonal Advanced Liftoff (%)	31.1	30.3	27.6	34.6	32.9
Tripedal Support (% of stride)	11.8	6.0	17.3	9.3	9.4
Bilateral Support (% of stride)	52.0	54.7	44.9	60.0	53.8
Diagonal Support (% of stride)	36.2	39.3	37.8	30.7	29.9
Unipedal Support (% of stride)	0.0	0.0	0.0	0.0	6.8
Front Contralateral Suspension (%)	0.0	0.0	0.0	0.0	6.8
Hind Contralateral Suspension (%)	0.0	0.0	0.0	0.0	0.0
Four-Limb Suspension (% of stride)	0.0	0.0	0.0	0.0	0.0
Intergascular Angle at Placement (°)	40.0	40.0	40.0	50.0	40.0
Stride Length (m)	1.60	1.63	1.80	1.89	2.21
Stride Length/Horse Height Ratio	1.21	1.22	1.18	1.24	1.44
Distance Between Diagonal Steps (cm)	40.7	42.6	43.9	35.0	49.5
Distance Between Ipsilateral Steps (cm)	14.0	13.3	15.3	29.9	30.5
Ipsilateral Overstep/Stride Length	0.09	0.08	0.09	0.16	0.22
Diagonal/Ipsilateral Step Distance	2.91	3.20	2.87	1.17	1.62
Average Interior Straddle (cm)	−2.6	−5.1	5.8	ca. -3.2	0.0
Average Foot Pair Lateral Offset (cm)	3.2	5.1	1.3	ca. 1.3	6.35
Foot Pair Lateral Offset/Hoof Width	0.24	0.42	0.09	0.10	0.42

Table S3.
Temporal and Linear Parameters of the Slow Rack or Tölt (Horses 1, 8–10) and Medium Rack (Horses 5 and 10) (Original Data).

Traits	Horse Numbers						
	8	**10**	**11**	**13**	**15**	**16**	**17**
Velocity (m/s)	3.88	3.75	3.65	ca. 3.20	ca. 3.90	ca. 4.40	ca. 6.80
Stride Duration (s)	0.58	0.59	0.55	0.50	0.51	0.50	0.49
Stride Frequency (s^{-1})	1.72	1.69	1.82	2.00	1.96	2.00	2.04
Front Stance Phase (% of stride)	46.1	48.3	50.0	40.5	40.1	42.0	38.3
Hind Stance Phase (% of stride)	53.0	56.2	50.0	41.5	50.0	41.0	42.9
Average Stance Phase (% of stride)	49.6	50.4	50.0	41.0	45.0	41.5	40.6
Front Stance/Hind Stance Ratio	0.87	0.92	1.00	0.98	0.80	1.02	0.89
Time Between Ipsilateral Steps (s)	0.12	0.13	0.11	0.11	0.11	0.11	0.11
Time Between Diagonal Steps (s)	0.17	0.17	0.17	0.16	0.15	0.14	0.14
Ipsilateral/Diagonal Step Time Ratio	0.74	0.75	0.65	0.72	0.71	0.82	0.75
Ipsilateral Swing Phase Overlap (%)	67.3	65.2	60.6	65.3	77.1	59.9	70.4
Ipsilateral Stance Phase Overlap (%)	59.8	59.0	60.6	49.1	58.5	45.2	49.8
Overall Ipsilateral Overlap (%)	63.6	62.1	60.6	57.2	67.8	52.5	60.1
Swing/Stance Phase Overlap Ratio	1.13	1.11	1.00	1.33	1.32	1.32	1.41
Lateral Advanced Placement (%)	21.3	21.4	19.7	21.0	20.8	22.5	21.4
Lateral Advanced Liftoff (% of stride)	15.2	16.7	19.7	21.5	12.9	23.0	17.3
Diagonal Advanced Placement (%)	28.7	28.6	30.0	29.0	29.2	27.5	28.6
Diagonal Advanced Liftoff (%)	34.3	32.9	30.3	32.0	38.6	25.5	33.7
Tripedal Support (% of stride)	7.0	9.4	0.0	0.0	0.0	0.0	0.0
Bilateral Support (% of stride)	57.4	53.8	60.6	58.0	58.4	55.0	57.1
Diagonal Support (% of stride)	30.4	29.9	39.4	19.0	22.8	31.0	18.4
Unipedal Support (% of stride)	5.2	6.8	0.0	23.0	18.8	14.0	24.5
Front Contralateral Suspension (%)	5.2	6.8	0.0	28.0	13.9	14.0	20.4
Hind Contralateral Suspension (%)	0.0	0.0	0.0	21.0	11.9	17.0	22.4
Four-Limb Suspension (% of stride)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Intergascular Angle at Placement (°)	40.0	40.0	40.0	40.0	40.0	45.0	45.0
Stride Length (m)	2.25	2.21	2.01	NA	NA	NA	NA
Stride Length/Horse Height Ratio	1.70	1.44	1.38	NA	NA	NA	NA
Distance Between Diagonal Steps (cm)	1.9	30.5	21.6	NA	NA	NA	NA
Distance Between Ipsilateral Steps (cm)	88.9	49.5	45.7	NA	NA	NA	NA
Ipsilateral Overstep/Stride Length	0.40	0.22	0.23	NA	NA	NA	NA
Diagonal/Ipsilateral Step Distance	0.02	0.62	0.47	NA	NA	NA	NA
Average Interior Straddle (cm)	−6.4	0.0	−8.9	NA	NA	NA	NA
Average Foot Pair Lateral Offset (cm)	10.2	11.5	3.2	NA	NA	NA	NA
Foot Pair Lateral Offset/Hoof Width	0.80	0.76	0.22	NA	NA	NA	NA

Table S4.
Temporal and Linear Parameters of the Fast Rack or Tölt (Horses 8, 10–11, 13, 15–17) (Original Data).

Traits	Horse Numbers						
	1	**4**	**9**	**13**	**14**	**19**	**20**
Velocity (meters/second)	3.08	2.95	4.18	ca. 3.20	ca. 5.70	ca. 4.60	ca. 2.50
Stride Duration (seconds)	0.64	0.75	0.52	0.66	0.50	0.55	0.67
Stride Frequency (strides per second)	1.56	1.33	1.92	1.52	2.00	1.82	1.49
Front Stance Phase (% of stride)	51.2	54.3	41.0	50.4	37.5	38.2	48.9
Hind Stance Phase (% of stride)	50.0	54.0	43.8	50.0	41.0	47.3	54.5
Average Stance Phase (% of stride)	50.6	54.2	42.4	50.2	39.2	42.7	51.7
Front Stance/Hind Stance Ratio	1.02	1.01	0.93	1.01	0.91	0.81	0.90
Time Between Ipsilateral Steps (s)	0.10	0.10	0.08	0.09	0.09	0.09	0.11
Time Between Diagonal Steps (s)	0.22	0.28	0.18	0.24	0.16	0.18	0.22
Ipsilateral/Diagonal Step Time Ratio	0.46	0.36	0.44	0.39	0.54	0.51	0.51
Ipsilateral Swing Phase Overlap (%)	68.4	71.0	78.0	72.0	75.5	88.8	72.7
Ipsilateral Stance Phase Overlap (%)	68.4	75.3	82.4	72.0	57.4	64.6	64.0
Overall Ipsilateral Overlap (%)	68.4	73.1	79.4	72.0	66.5	76.7	67.3
Swing/Stance Phase Overlap Ratio	1.00	0.94	0.95	1.00	1.32	1.37	1.14
Lateral Advanced Placement (%)	15.7	13.3	15.2	14.0	17.5	16.8	16.9
Lateral Advanced Liftoff (% of stride)	15.7	13.3	12.4	14.0	14.0	5.9	10.9
Diagonal Advanced Placement (%)	34.3	36.7	34.8	36.0	32.5	33.2	33.1
Diagonal Advanced Liftoff (%)	34.3	36.7	37.6	36.0	34.5	44.1	37.6
Tripedal Support (% of stride)	0.0	16.0	0.0	0.0	0.0	0.0	9.0
Bilateral Support (% of stride)	68.5	65.3	69.5	72.0	65.0	66.4	62.2
Diagonal Support (% of stride)	31.5	18.7	12.4	28.0	13.0	6.4	24.8
Unipedal Support (% of stride)	0.0	0.0	18.1	0.0	22.0	27.3	0.0
Front Contralateral Suspension (%)	0.0	0.0	19.0	0.0	26.0	27.3	0.0
Hind Contralateral Suspension (%)	0.0	0.0	14.3	0.0	22.4	13.6	0.0
Four-Limb Suspension (% of stride)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Intergascular Angle at Placement (°)	40.0	50.0	50.0	40.0	40.0	50.0	40.0
Stride Length (m)	1.97	2.21	2.17	NA	NA	NA	NA
Stride Length/Horse Height	1.31	1.36	1.62	NA	NA	NA	NA
Distance Between Diagonal Steps (cm)	13.0	16.5	12.7	NA	NA	NA	NA
Distance Between Ipsilateral Steps (cm)	61.5	50.2	70.5	NA	NA	NA	NA
Ipsilateral Overstep/Stride Length Ratio	0.31	0.23	0.33	NA	NA	NA	NA
Diagonal/Ipsilateral Step Distance Ratio	0.21	0.33	0.18	NA	NA	NA	NA
Average Interior Straddle (cm)	2.0	−1.3	−3.9	NA	NA	NA	NA
Average Foot Pair Lateral Offset (cm)	4.0	ca. 15.0	14.0	NA	NA	NA	NA
Foot Pair Lateral Offset/Hoof Width	0.32	0.88	1.16	NA	NA	NA	NA

Table S5.
Temporal and Linear Parameters of the Stepping Pace (Horses 1, 4, 9, 13–14, and 19–20) (Original Data).

Traits	Horse Number	
	1	6
Velocity (meters/second)	3.25	3.51
Stride Duration (seconds)	0.64	0.60
Stride Frequency (strides per second)	1.56	1.67
Front Stance Phase (% of stride)	50.0	50.0
Hind Stance Phase (% of stride)	46.9	55.0
Average Stance Phase (% of stride)	48.4	52.5
Front Stance/Hind Stance Ratio	1.07	0.91
Time Between Ipsilateral Steps (seconds)	0.07	0.03
Time Between Diagonal Steps (seconds)	0.25	0.27
Ipsilateral/Diagonal Step Time Ratio	0.26	0.11
Ipsilateral Swing Phase Overlap (%)	76.4	100.0
Ipsilateral Stance Phase Overlap (%)	85.3	90.9
Overall Ipsilateral Overlap (% of stride)	80.4	95.4
Swing/Stance Phase Overlap Ratio	0.90	1.10
Lateral Advanced Placement (% of stride)	10.2	5.0
Lateral Advanced Liftoff (% of stride)	11.8	0.0
Diagonal Advanced Placement (% of stride)	39.8	45.0
Diagonal Advanced Liftoff (% of stride)	33.8	50.0
Tripedal Support (% of stride)	4.7	10.0
Bilateral Support (% of stride)	74.0	90.0
Diagonal Support (% of stride)	21.3	0.0
Unipedal Support (% of stride)	0.0	0.0
Front Contralateral Suspension (% of stride)	0.0	0.0
Hind Contralateral Suspension (% of stride)	0.0	0.0
Four-Limb Suspension (% of stride)	0.0	0.0
Intergascular Angle at Placement (°)	35.0	40.0
Stride Length (m)	2.08	2.11
Stride Length/Horse Height (at withers)	1.39	1.59
Distance Between Diagonal Steps (cm)	15.0	17.2
Distance Between Ipsilateral Steps (cm)	65.0	59.7
Ipsilateral Overstep/Stride Length Ratio	0.31	0.28
Diagonal/Ipsilateral Step Distance Ratio	0.23	0.29
Average Interior Straddle (cm)	ca. 5.0	−2.3
Average Foot Pair Lateral Offset (cm)	ca. 17.2	10.2
Foot Pair Lateral Offset/Hoof Width Ratio	1.38	0.86

Table S6.
Temporal Parameters of the Slow Pace (Horses 1, 6) (Original Data).

Traits	Horse Number		
	6	**7**	**18**
Velocity (meters/second)	ca. 2.90	3.70	ca. 3.30
Stride Duration (seconds)	0.59	0.67	0.60
Stride Frequency (strides per second)	1.69	1.49	1.67
Front Stance Phase (% of stride)	48.3	48.9	51.3
Hind Stance Phase (% of stride)	59.4	54.9	57.9
Average Stance Phase (% of stride)	53.8	51.9	54.6
Front Stance/Hind Stance Ratio	0.81	0.89	0.88
Time Between Ipsilateral Steps (seconds)	0.25	0.27	0.20
Time Between Diagonal Steps (seconds)	0.04	0.06	0.10
Ipsilateral/Diagonal Step Time Ratio	5.88	4.36	2.08
Ipsilateral Swing Phase Overlap (% of stride)	23.6	20.2	37.5
Ipsilateral Stance Phase Overlap (%)	28.2	25.6	41.7
Overall Ipsilateral Overlap (% of stride)	25.9	22.9	39.6
Swing/Stance Phase Overlap Ratio	0.84	0.79	0.90
Lateral Advanced Placement (% of stride)	42.7	40.7	31.7
Lateral Advanced Liftoff (% of stride)	32.5	35.4	27.1
Diagonal Advanced Placement (% of stride)	7.3	9.3	16.2
Diagonal Advanced Liftoff (% of stride)	16.7	14.2	27.5
Tripedal Support (% of stride)	13.7	10.4	13.3
Bilateral Support (% of stride)	14.5	18.7	32.5
Diagonal Support (% of stride)	63.2	70.9	54.2
Unipedal Support (% of stride)	8.5	0.0	0.0
Front Contralateral Suspension (% of stride)	14.5	0.0	0.0
Hind Contralateral Suspension (% of stride)	0.0	0.0	0.0
Four-Limb Suspension (% of stride)	0.0	0.0	0.0
Intergascular Angle at Placement (°)	40.0	40.0	40.0
Stride Length (m)	NA	2.47	NA
Stride Length/Horse Height (at withers)	NA	1.50	NA
Distance Between Diagonal Steps (cm)	NA	90.2	NA
Distance Between Ipsilateral Steps (cm)	NA	ca. -2.5	NA
Ipsilateral Overstep/Stride Length Ratio	NA	−0.01	NA
Diagonal/Ipsilateral Step Distance Ratio	NA	−36.08	NA
Average Interior Straddle (cm)	NA	15.9	NA
Average Foot Pair Lateral Offset (cm)	NA	ca. 5.7	NA
Foot Pair Lateral Offset/Hoof Width	NA	0.39	NA

Table S7.
Temporal and Linear Parameters of the Fox Trot (Horses 6, 7, and 18) (Original Data).

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Chapter 9

Microbial Diversity and Community Dynamics in the Intestines of Broiler Chicken Raised in an Open-Sided House

Waleed Al-Marzooqi

Abstract

The intestinal microbiota of the chicken plays a central role in enhancing nutrient absorption and affecting both host performance, health and immunity. This study was conducted to assess the relative abundance of bacteria microflora in different segments of the gastrointestinal tract (duodenum, jejunum, ileum, and cecum) of broiler chicken raised in an open-sided house. One hundred fifty-one-day-old chicks of Cobb 500 broiler chickens were raised in an open-sided house fed a standard non-medicated corn-soybean meal diet from day 0–35 days of age. The study showed a distinctive difference in the bacterial community between each region of intestinal segments and the diversity of the bacterial community changed as the chicken aged. In addition, Lactobacillales were the dominant 16S rDNA sequences in the duodenum, jejunum, and ileum libraries, whereas Clostridiales were the dominant 16S rDNA sequences in the cecum libraries. The bacterial microbiota relative abundance differed significantly ($p < 0.05$) across different intestinal segments. In conclusion, each region developed its own bacterial community and the relative abundances of the bacterial community were quite different. Based on the composition of the microbial community, future gut modulation with beneficial bacteria, such as probiotics, may benefit the host.

Keywords: broiler chicken, 16S rDNA, intestine, gut microbiota, open-sided house

1. Introduction

Many studies have used sequencing technologies to characterize the microbial communities that colonies the gastrointestinal tracts of chickens and to characterize the development of these communities over time [1]. It is well known that the chicken gut microbiota influences the host gut development, growth performance, and overall health [2, 3]. Different factors, such as diet and bird age, have a strong influence on the diversity and composition of the intestinal microbiome in chickens, which has grown in complexity and richness as chickens have grown [3, 4]. Each region of the gastrointestinal tract (GIT) develops its own distinct bacterial community, and the structure of the microflora gets more complicated and varies as chickens age, position in the digestive system, feed, breed, and environment change [5–8].

Several studies have shown the beneficial effect of gut microbiota on the physiological, metabolic, immunological, digestion, and nutrient absorption of the host [6]. Evaluation of the bacterial community and intestinal development of different genetic lines of chickens has become a recent point of interest [9]. A greater understanding of the chicken gut function and microbiology will provide a new opportunity for the improvement of broiler chicken health and production raised in an open-sided house.

The use of molecular approaches, which involve examining the structure of bacterial communities by detecting the distinguishing features of microbial DNA isolated from community samples, has solved the problems associated with microbe culture [10, 11]. Using these methods, researchers discovered that 90% of the bacteria in the chicken GIT belong to previously undiscovered species [12]. Furthermore, metagenomics (a nonculture-based technique) was established, allowing researchers to examine microbial communities in various habitats in-depth [13]. Metagenomic analysis has provided important information on microbial community alterations and succession [13].

Understanding the taxonomic composition of the bacterial community of the gastrointestinal tract, diversity and succession will permit detecting disruption in the microbiota. This information is crucial, as it may allow for the manipulation of intestinal flora to improve intestinal health and overall bird performance. The objective of this study was to use 16S rDNA-based analysis to analyze the relative abundance, diversity, and changes with age in the microbial community detected in different sections of the gut of broiler chickens.

2. Materials and methods

All experimental work was carried out at the Agricultural Experiment Station's Poultry Research Unit in compliance with the experimental unit's animal welfare policy and the requirements of procedures involving animals/birds and their care. The Animal Research Ethics Board at Sultan Qaboos University approved the research project.

2.1 Birds management and diet

One-day-old chicks (Cobb 500) were obtained from a commercial hatchery and screened upon arrival to ensure that no abnormalities or symptoms of the disease were present. Throughout the experiment, standard operating procedures for

Figure 1.
Photograph of an open-sided poultry house used in the current experiment.

Ingredients (%)	Amount
Corn	56.18
Soybean meal (48%)	37.4
Vegetable Oil	3
Monocalcium Phosphate	1.751
Limestone	0.853
Salt	0.290
Vitamin and Mineral Premix[1]	0.325
DL-Methionine	0.201
Total	100
Calculated Analysis	—
ME (kcal/kg)	3095
Crude Protein (%) Available phosphorus (%)	22.5 0.45
Lysine (%)	1.15
Calcium (%)	0.9
Methionine (g)	0.53

[1]1 kg of premix used contains a blend of Vitamin and Mineral all balanced according to the breed recommendation.

Table 1.
Composition of the conventional diet used in the experiment.

broiler house management [14] were followed. Before the experiment, the Open-sided house unit, cages, feeders, and drinks were fumigated to clean and disinfect them. In addition, strong cleanliness and biosecurity measures were implemented. The open-sided house was constructed from a galvanized iron shed with profiled steel shed roofing that was naturally ventilated. On all sides, chicken mesh panels and a one-meter-high block work protection were installed. It included four sets of electric wall fans to help circulate the air. Shade cloths were used to screen direct sun rays during midday (**Figure 1**). Six birds were randomly assigned to each of 15 suspended wire cages (62 × 62 × 37 cm) such that all cages had nearly a similar average initial weight. Feed was available ad libitum and the composition of the experimental diet is presented in **Table 1**. The house temperature was maintained at 33°C on day 1 and reduced by 3°C each week to reach a constant 22°C. The lighting program was 23 L—1D. There were 15 replicates with each replicate cage containing six birds (a total of 90 birds). Birds per replicate combinations were randomly allocated.

2.2 Sample collection

One bird per cage was chosen at random at the ages of 5, 15, 25, and 35 days. The birds were chosen, marked, and maintained in their cages until they were euthanized, based on the weight of their bodies. Each cage's birds with the closest body weight to the average were chosen, labeled, and euthanized accordingly. Then birds were injected intramuscularly with a xylazine-ketamine combination containing 5 mg xylazine (Ilium Xylazil-20-Xylazine 20 mg/mL, as hydrochloride) and 25 mg ketamine (Ketamine Injection-Ketamine 100 mg/mL as hydrochloride) to put the birds into a deep plan of sedation and anesthesia. Both xylazine–ketamine were supplied by Troy Laboratories Pty Limited, Glendenning, Australia. The bird was euthanized via cervical dislocation once it was totally immobilized. Then, at the

bottom of the breastbone, an incision was made and a huge V shape was carved toward the head. The abdominal cavity was opened at the apex of the V form, taking care not to burst the intestine below. The small intestine was carefully pushed out of the abdominal cavity till the ileal-caecal-colonic junction was observed once a large enough opening had been formed. The duodenum (from the gizzard to the bile and pancreatic ducts), jejunum (from the ducts to the yolk stalk), ileum (from the yolk stalk to the ileocecal junction), and caecum (two horns) were distinguished, separated, and cleaned with 70% alcohol wipes.

The mid portions of the duodenum, jejunum, ileum, and ceca were cut into 4 cm long sections (including digesta). After each bird, the dissecting instruments were cleaned with 70% ethanol. The entire procedure of collecting intestinal contents took less than 30 minutes and was completed on a thoroughly cleaned workbench.

The contents of the four segments of the intestine were collected and placed in a sterile 15-mL conical tube with labels. The samples were immediately placed on ice and transferred to the laboratory, where they were kept in an 80°C freezer until they were analyzed. The analysis of all samples began 2 weeks after the trial ended on day 35. All of the samples were taken under identical conditions.

2.3 DNA extraction

Total DNA was extracted from the contents of each intestinal segment (duodenum, jejunum, ileum, and cecum) according to the manufacturer's instructions using a QIAamp DNA Stool Mini Kit (QIAGEN, CA, Hamburg, Germany). The optical density of the 16 DNA samples was measured using a Nano-Drop 2000 (Thermo Electron Corporation, Waltham, MA, USA) at wavelengths of 260 and 280 nm to assess their integrity. Using 1.0% agarose gel electrophoresis (including ethidium bromide), the integrity of the DNA extracts was visually evaluated.

2.4 Polymerase chain reaction amplicon production and high-throughput sequencing

The variable regions V3-V4 of the 16S rDNA gene were amplified and sequenced. For the PCRs, 5 mM of each primer, 10 ng of DNA template, 4 liters of 1 FastPfu buffer, 2.5 mM dNTPs, and 0.4 liters of FastPfu polymerase in triplicate in a total volume of 20 liters were used (TransGen Biotech, Beijing, China). The PCR conditions were as follows: Denaturation at 95°C for 2 minutes, then 25 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing at 55°C, and 30 seconds extension at 72°C, followed by a final extension at 72°C for 5 minutes. Amplicons produced from different intestinal luminal content samples were sent to a commercial company (BGI Genomic Lab, Tai Po Industrial Zone, New Territories, Hong Kong, China) for sequencing on the Illumina MiSequencing platform.

2.5 Sequencing analysis

To get operational sequences, all of the raw sequences obtained from Illumina Miseq were first filtered for quality control. The sequences were checked for quality and analyzed using the software Quantitative Insights into Microbial Ecology (QIIME, v1.8.0) [15]. FLASH [16] was used to combine the paired-end readings from the DNA fragments. Read trimming and identification of V3-V4 sequences were performed on the sequencing data, and a group of sequences with 97% identity was identified as an operational taxonomic unit (OTU). To cluster operational taxonomic units, the UCLUST [17] clustering approach was applied. At a cutoff of 97%, the determined OTUs were allocated to different

taxonomic levels (phylum, class, genus, and families). Furthermore, the Shannon and Simpson diversity indices, abundance-based coverage estimators (ACE), Chao 1 richness, and coverage percentage were all calculated by Good's method. Also, clustered OTUs were used to construct the rarefaction curves.

2.6 Data analyses and bioinformatics

The QIIME and R packages were used for bioinformatics and statistical studies (v3.1.1). To determine the relative abundance and diversity of the sequences, the alpha-diversity indices (ACE, Chaol, Shannon, and Simpson index) were calculated. To examine whether taxonomic categories were significantly different across groups of samples based on intestine segments and age period, Metastats and R package (v3.1.1) [18] were used. At p 0.05, the differences were considered significant. A Benjamini–Hochberg false discovery rate correction (Function "p. adjust" in the stats package of R (v3.1.1)) was used to alter the obtained p-value.

3. Results

The diversity of bacterial populations detected in distinct intestinal segments was highlighted by data acquired by molecular detection and bioinformatics analysis. The 16S rDNA study produced a large amount of data that is beyond the scope of this article. As a result, the current study's findings have been confined to the most quantitatively significant Classes/Orders of bacteria.

3.1 Sequencing overview

A total of 128 samples were obtained from a combination of four intestinal segments—(duodenum, jejunum, ileum, and caecum) and four age periods—(day 5, day 15, day 25, and day 35) with n = 8 per group and subsequently sequenced to generate V3-V4 of the 16S rDNA gene profiles A total of 1179,68 sequences were obtained with the number of sequences ranging from 58,498 to 112,785 and clustered into 14 to 133 OTUs for each sample, resulting in a total of 253 OTUs for all samples at the 97% sequence similarity value. The microbial complexity and microbial community composition and abundance of water are summarized in their respective sections below.

3.1.1 The microbial complexity

The alpha-diversity indices were used to evaluate the microbial complexity in the duodenum, jejunum, ileum, and cecum (ACE index, Chao1 index, Simpson index, and Shannon index) **Table 2** The Chao1 was used to quantify species diversity, while Simpson's and Shannon's indexes were used to evaluate species richness. When comparing the means of the indices, there was a substantial difference across intestinal regions throughout age periods.

3.2 Microbial composition of the duodenum

Bacteria classified according to their respective Class and Order, found in the duodenum of broiler chickens at different ages, are presented in **Table 3**. Sixteen bacterial microbiota at the Order level were found in the duodenum. Of the 30,173 reads, Lactobacillales were the most abundant Order, from the Class Bacilli, at 78.73% of the total number of sequences. Clostridiales, a representative Order

	ACE		Chao1		Simpson		Shannon	
Intestinal Segment	**Mean**	**STD**	**Mean**	**STD**	**Mean**	**STD**	**Mean**	**STD**
Duodenum	133.20	14.12	111.36	21.42	0.319	0.183	1.95	1.03
Jejunum	69.92	29.57	64.28	28.40	0.377	0.162	1.55	0.535
Ileum	74.44	42.85	70.16	38.14	0.614	0.342	1.09	1.09
Cecum	137.14	35.00	137.84	34.11	0.258	0.148	2.25	0.605
P-value	0.0252		0.0527		0.2285		0.1377	

The differences were considered to be significant at P < 0.05. These results emanate from our own experiment.

Table 2.
The average alpha-diversity indices (ACE, Chao1, Simpson, and Shannon indices) of the data distribution.

		Abundance of sequence (no. of sequence [%]) at day:			
Class	**Order**	**Day5**	**Day15**	**Day25**	**Day35**
Actinobacteria	Actinomycetales	191 (2.32)	43 (0.53)	46 (0.53)	0 (0.00)
	Bifidobacteriales	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Coriobacteriia	Coriobacteriales	3 (0.04)	0 (0.00)	0 (0.00)	15 (0.28)
Bacteroidia	Bacteroidales	0 (0.00)	0 (0.00)	0 (0.00)	8 (0.15)
4C0d-2	YS2	0 (0.00)	0 (0.00)	2 (0.02)	175 (3.32)
Chloroplast	Streptophyta	20 (0.24)	34 (0.42)	293 (3.38)	0 (0.00)
Bacilli	Bacillales	98 (1.19)	5 (0.06)	14 (0.16)	3 (0.06)
	Lactobacillales	7679 (93.37)	8000 (98.80)	7771 (89.77)	304 (5.77)
Clostridia	Clostridiales	146 (1.78)	5 (0.06)	399 (4.61)	4675 (88.71)
Erysipelotrichi	Erysipelotrichales	2 (0.02)	0 (0.00)	0 (0.00)	42 (0.80)
Alphaproteobacteria	Caulobacterales	11 (0.13)	1 (0.01)	3 (0.03)	0 (0.00)
	Rhizobiales	21 (0.26)	3 (0.04)	11 (0.13)	0 (0.00)
Betaproteobacteria	Rhodocyclales	5 (0.06)	0 (0.00)	2 (0.02)	0 (0.00)
Epsilonproteobacteria	Campylobacterales	11 (0.13)	3 (0.04)	84 (0.97)	0 (0.00)
Gammaproteobacteria	Enterobacteriales	1 (0.01)	0 (0.00)	5 (0.06)	0 (0.00)
Mollicutes	RF39	0 (0.00)	0 (0.00)	0 (0.00)	32 (0.61)
Unclassified	Unclassified	0 (0.01)	0 (0.00)	0 (0.00)	6 (0.11)
Others (<0.5%)	Others (<0.5%)	1 (0.43)	0 (0.04)	0 (0.31)	0 (0.19)
Total		**8189**	**8094**	**8630**	**5260**

These results emanate from our own experiment.

Table 3.
The abundance of bacterial 16S rDNA sequences (n = **30,173***) identified from the* **duodenum** *microflora of cobb 500 broiler chicken.*

from the Class Clostridia, was the second most common Order accounting for 17.32% of the total number of sequences. At the Class level, Actinobacteria, and Chloroplast accounted for 0.93% and 1.15%, of the total number of sequences, respectively, at the Class level. Lactobacillales were the most dominant group across all age groups, accounting for 93.37% at day 5, 98.80% at day 15, and 89.77% at day 25 to 5.77% at day 35 of the sequences. Clostridiales were the second most

abundant, sequences fluctuated from 1.78% at day 5, 0.06% at day 15, 4.61% at day25, and 88.71% at day 35. Comparatively, Coriobacteriales, (member from Class Coriobacteriia), Bacteroidales, (Class, Bacteroidia), Bacillales, (member from Class Bacilli), Erysipelotrichales (Class, Erysipelotrichi), Rhizobiales (Class, Alphaproteobacteria), Rhodocyclales (Class, Betaproteobacteria), Enterobacteriales (Class, Gammaproteobacteria), and RF39 (member from Class Mollicutes) group-related sequences were detected at smaller percentages through all age periods.

3.3 Microbial composition of the jejunum

Bacteria classified according to their respective Class and Order, found in the jejunum of broiler chickens at different ages, are presented in **Table 4**. Sixteen bacterial microbiota at the Order level were found in the jejunum. Of the 28,646 reads, Lactobacillales were the most abundant Order, from the Class Bacilli, at 75.95% of the total sequences. Clostridiales, a representative Order from the Class Clostridia, was the most second Order accounted for 11.21% of the total sequences. At the Class level, only a few 4.46% Actinobacteria-related sequences were detected; these were related to Actinomycetales and Bifidobacteriales. Chloroplast, Erysipelotrichi, and Gammaproteobacteria at the Class level represented a small percentage of 2.32%, 1.55%, and 4.06%, respectively, of the total sequences. Across different age periods,

		Abundance of sequence (no. of sequence [%]) at day:			
Class	**Order**	**Day5**	**Day15**	**Day25**	**Day35**
Actinobacteria	Actinomycetales	23 (0.27)	154 (2.33)	8 (0.11)	0 (0.00)
	Bifidobacteriales	4 (0.05)	0 (0.00)	0 (0.00)	1090 (17.44)
Coriobacteriia	Coriobacteriales	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Bacteroidia	Bacteroidales	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
4C0d-2	YS2	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Chloroplast	Streptophyta	64 (0.75)	427 (6.45)	175 (2.39)	0 (0.00)
Bacilli	Bacillales	14 (0.17)	23 (0.35)	2 (0.03)	9 (9.99)
	Lactobacillales	7892 (93.09)	5695 (86.01)	6703 (91.45)	1466 (23.46)
Clostridia	Clostridiales	367 (4.33)	278 (4.20)	63 (0.86)	2504 (40.07)
Erysipelotrichi	Erysipelotrichales	13 (0.15)	0 (0.00)	1 (0.01)	429 (6.87)
Alphaproteobacteria	Caulobacterales	4 (0.05)	4 (0.06)	2 (0.03)	0 (0.00)
	Rhizobiales	29 (0.34)	17 (0.26)	12 (0.16)	0 (0.00)
Betaproteobacteria	Rhodocyclales	6 (0.07)	2 (0.03)	0 (0.00)	0 (0.00)
Epsilonproteobacteria	Campylobacterales	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Gammaproteobacteria	Enterobacteriales	51 (0.60)	1 (0.02)	361 (4.92)	751 (12.02)
Mollicutes	RF39	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Unclassified	Unclassified	0 (0.01)	0 (0.02)	0 (0.00)	0 (0.00)
Others (<0.5%)	Others (<0.5%)	1 (0.12)	1 (0.29)	0 (0.04)	0 (0.00)
Total		**8468**	**6602**	**7327**	**6249**

These results emanate from our own experiment.

Table 4.
The abundance of bacterial 16S rDNA sequences (n = **28,646***) identified from the* **jejunum** *microflora of cobb 500 broiler chicken.*

Lactobacillales were the most dominant group, representing 93.09% at day 5, 86.01% at day15, 91.45% at day 25 to 23.46% at day 35 of the sequences. Clostridiales were the second most abundant, sequences fluctuated from 4.33% at day 5, 4.02% at day 15, 0.86% at day 25, and 40.07% at day 35. Relatively, Bifidobacteriales, (Class, Actinobacteria), Streptophyta (Class, Chloroplast), Bacillales (Class, Bacilli), Erysipelotrichales (Class, Erysipelotrichia), Rhizobiales (member from Class, Alphaproteobacteria), Rhodocylales (Class, Betaproteobacteria) and Enterobacteriales (member from Class, Gammaproteobacteria) group-related sequences were detected at lower levels across age periods.

3.4 Microbial composition of the ileum

Bacteria classified according to their respective Class and Order, found in the ileum of broiler chickens at different ages, are presented in **Table 5**. Sixteen bacterial microbiota at the Order level were found in the ileum. Of the 30,961 reads, Lactobacillales were the most abundant Order, from the Class Bacilli, at 51.36% of the total sequences. Clostridiales, a representative Order from the class Clostridia, was the most second Order accounted for 18.35% of the total sequences. At the Class level, only a few 0.90% Actinobacteria-related sequences were detected; these were related to Actinomycetales and Bifidobacteriales. At the Class level, Coriobacteriia,

		Abundance of sequence (no. of sequence [%]) at day:			
Class	**Order**	**Day5**	**Day15**	**Day25**	**Day35**
Actinobacteria	Actinomycetales	54 (0.63)	217 (2.40)	1 (0.01)	0 (0.00)
	Bifidobacteriales	2 (0.02)	3 (0.03)	0 (0.00)	0 (0.00)
Coriobacteriia	Coriobacteriales	0 (0.00)	0 (0.00)	0 (0.00)	87 (1.46)
Bacteroidia	Bacteroidales	2 (0.02)	3 (0.03)	0 (0.00)	40 (0.67)
4C0d-2	YS2	0 (0.00)	0 (0.00)	0 (0.00)	7 (0.12)
Chloroplast	Streptophyta	7 (0.08)	466 (5.16)	17 (0.23)	0 (0.00)
Bacilli	Bacillales	26 (0.30)	48 (0.53)	1 (0.01)	0 (0.00)
	Lactobacillales	8432 (98.69)	8174 (90.59)	7466 (99.63)	3 (0.05)
Clostridia	Clostridiales	12 (0.14)	51 (0.57)	8 (0.11)	5659 (95.17)
Erysipelotrichi	Erysipelotrichales	0 (0.00)	1 (0.01)	0 (0.00)	137 (2.30)
Alphaproteobacteria	Caulobacterales	1 (0.01)	5 (0.06)	0 (0.00)	0 (0.00)
	Rhizobiales	2 (0.02)	13 (0.14)	0 (0.00)	0 (0.00)
Betaproteobacteria	Rhodocyclales	1 (0.01)	0 (0.00)	0 (0.00)	0 (0.00)
Epsilonproteobacteria	Campylobacterales	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Gammaproteobacteria	Enterobacteriales	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.03)
Mollicutes	RF39	0 (0.00)	0 (0.00)	0 (0.00)	11 (0.18)
Unclassified	Unclassified	0 (0.00)	0 (0.02)	0 (0.00)	0 (0.00)
Others (<0.5%)	Others (<0.5%)	0 (0.06)	2 (0.44)	0 (0.01)	0 (0.00)
Total		**8539**	**8983**	**7493**	**5946**

These results emanate from our own experiment.

Table 5.
The abundance of bacterial 16S rDNA sequences (n = **30,961***) identified from the* **ileum** *microflora of cobb 500 broiler chicken.*

Bacteroidia, and Erysipelotrichi represented small percentages of 0.32%, 0.14%, and 0.44%, respectively, of the total sequences. Across different age periods, Lactobacillales were the most dominant group, representing 98.69% at day 5, 90.59% at day 15, 99.63% at day 25 to 0.05% at day 35 of the sequences. Clostridiales were the second most abundant, sequences fluctuated from 0.14% at day 5, 0.57% at day 15, 0.11% at day 25, and 95.17% at day 35. Relatively, Actinomycetales (Class, Actinobacteria), Coribacteriales, (member from Class Coriobacteria), Bacteroidales, (Class, Bacteroidia), Streptophyta (Class, Chloroplast), Bacillales (Class, Bacilli), Erysipelotrichales (Class, Erysipelotrichi) group-related sequences were detected at smaller percentages across all age periods.

3.5 Microbial composition of the cecum

Bacteria classified according to their respective Class and Order, found in the Cecum of broiler chickens at different ages, are presented in **Table 6**. Sixteen bacterial microbiota at the Order level were found in the cecum. Of the 27,842 reads, Clostridiales, were the most abundant Order, a representative Order from

		Abundance of sequence (no. of sequence [%]) at day:			
Class	**Order**	**Day5**	**Day15**	**Day25**	**Day35**
Actinobacteria	Actinomycetales	53 (0.93)	1505 (21.89)	92 (1.30)	0 (0.00)
	Bifidobacteriales	4 (0.07)	30 (0.44)	1 (0.01)	0 (0.0)
Coriobacteriia	Coriobacteriales	21 (0.37)	25 (0.36)	0 (0.00)	114 (1.39)
Bacteroidia	Bacteroidales	32 (0.56)	35 (0.51)	2 (0.03)	2010 (24.57)
4C0d-2	YS2	13 (0.23)	9 (0.13)	0 (0.00)	27 (0.33)
Chloroplast	Streptophyta	92 (1.62)	1101 (16.01)	855 (12.06)	0 (0.00)
Bacilli	Bacillales	33 (0.58)	224 (3.26)	22 (0.31)	131 (1.61)
	Lactobacillales	1480 (25.99)	1134 (16.49)	925 (13.05)	11 (0.13)
Clostridia	Clostridiales	3851 (62.55)	2613 (38.00)	5144 (72.55)	5880 (71.87)
Erysipelotrichi	Erysipelotrichales	24 (0.42)	24 (0.35)	2 (0.03)	6 (0.07)
Alphaproteobacteria	Caulobacterales	14 (0.25)	45 (0.65)	2 (0.03)	0 (0.0)
	Rhizobiales	31 (0.54)	86 (1.25)	40 (0.56)	0 (0.00)
Betaproteobacteria	Rhodocyclales	38 (0.67)	42 (0.610)	4 (0.06)	0 (0.00)
Epsilonproteobacteria	Campylobacterales	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.0()
Gammaproteobacteria	Enterobacteriales	8 (0.14)	3 (0.04)	1 (0.01)	0 (0.00)
Mollicutes	RF39	1 (0.02)	0 (0.00)	0 (0.00)	1 (0.01)
Unclassified	Unclassified	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Others (<0.5%)	Others (<0.5%)	0 (0.78)	0 (1.31)	0 (0.53)	0 (0.00)
Total		**5695**	**6876**	**7090**	**8181**

These results emanate from our own experiment.

Table 6.
The abundance of bacterial 16S rDNA sequences (n = **27,842***) identified from the* **cecum** *microflora of cobb 500 broiler chicken.*

the Class Clostridia, at 62.81% of the total sequences. Lactobacillales, which was the most second Order from the Class Bacilli, accounted for 12.75% of the total sequences. At the Class level, only a few 6.1% Actinobacteria-related sequences were detected; these were related to Actinomycetales and Bifidobacteriales. At the Class level, Bacteroidia and Alphaproteobacteria represented small percentages of 7.47% and 0.79%, respectively, of the total sequences. Across different age periods, Clostridiales were the most dominant group, representing 62.55% at day 5, 38% at day 15, 72.55% at day 25 to 71.87% at day 35 of the sequences, Lactobacillales were the second most abundant, sequences fluctuated from 25.99% at day 5, 16.49% at day 15, 13.05% at day 25 and 0.13% at day 35. Smaller percentage of sequences for Bacteroidales, (Class, Bacteroidia), were observed day 5: 0.56%, day 15: 0.51%, day 25: 0.03% and day 35: 24.57%. Relatively, Coriobacteriales (member from Class Coriobacteriia), Bacillales (member from Class Bacilli), Erysipelotrichales (member from Class, Erysipelotrichi), Rhizobiales (member from Class, Alphaproteobacteria), and Rhodocyclales (Class, Betaproteobacteria) group-related sequences were detected at lower levels across age periods.

3.6 Differences of microbial communities among samples from different intestinal segments of broiler chickens

Table 7 shows the p value distribution of 16S rDNA gene sequence libraries used to compare relative abundance differences of microbial communities between

	P- Value					
Order	**Duodenum-Jejunum**	**Duodenum-Ileum**	**Ileum-Jejunum**	**Caecum-Duodenum**	**Caecum-Ileum**	**Caecum-Jejunum**
Actinomycetales	0.340	0.363	0.011	0.099	0.093	0.030
Bifidobacteriales	0.533	0.015	0.497	0.252	0.495	0.485
Coriobacteriales	0.917	0.255	0.038	0.406	0.032	0.013
Bacteroidales	0.888	0.392	0.335	0.315	0.352	0.275
YS2	0.782	0.567	0.216	0.739	0.204	0.214
Streptophyta	0.031	0.092	0.017	0.816	0.014	0.024
Bacillales	0.606	0.051	0.940	0.100	0.863	0.578
Lactobacillales	0.545	0.926	0.000	0.808	0.001	0.000
Clostridiales	0.781	0.813	0.001	0.934	0.000	0.001
Erysipelotrichales	0.715	0.103	0.070	0.617	0.043	0.040
Caulobacterales	0.632	0.048	0.009	0.206	0.122	0.002
Rhizobiales	0.645	0.810	0.002	0.794	0.047	0.019
Rhodocyclales	0.395	0.188	0.121	0.406	0.407	0.058
Campylobacterales	0.574	0.601	0.371	0.547	0.263	0.318
Enterobacteriales	0.325	0.591	0.572	0.387	0.446	0.804
RF39	1.000	1.000	0.111	—	0.056	0.104
Unclassified	0.680	0.500	0.635	0.121	0.821	0.318

These results emanate from our own experiment.

Table 7.
P-value distribution of 16S rDNA gene sequence libraries compared the abundance differences of microbial communities among samples from the different segments for cobb 500 broiler chicken.

samples from different intestinal regions of broiler chickens. The composition of the bacterial microbiota in the duodenum-jejunum, duodenum-ileum, cecum-duodenum, cecum-ileum, and cecum-jejunum differed considerably (p0.05) in statistical comparisons of the libraries, implying that each region established its own bacterial community. The relative abundance of Actinomycetales at different intestinal segment differed significantly ($p<0.05$). In the duodenum, jejunum, and ileum libraries, Lactobacillales were the most common 16S rDNA sequences, while Clostridiales were the most common 16S rDNA sequences in the cecum libraries.

3.6.1 Differences of microbial communities among samples of different age groups

Table 8 presents the p value distribution of 16S rDNA gene sequence libraries used to compare quantitative differences in microbial communities between samples from broiler chickens of various age groups. Statistical analyses of the libraries revealed that the microbial composition at different age groups—day 5-day 15, day 5-day 25, day 5-day 35, day 15-day 25, day 15-day 35, and day 25-day 35 had no significant changes ($p > 0.05$).

3.7 The taxonomic composition distribution of the bacterial Community in Intestinal Segments at the order-level

The diversity of the bacterial population in the intestinal segments of broiler chickens shifted from one age period to the next, as shown in **Figure 2**. Species with an abundance of less than 0.5% across all samples were labeled "Unclassified." The duodenum, jejunum, and ileum had a larger abundance of Lactobacillales, and the

	P- Value					
Order	**Day5-Day15**	**Day5-Day25**	**Day5-Day35**	**Day15-Day25**	**Day15-Day35**	**Day25-Day35**
Actinomycetales	0.869	0.908	0.904	0.458	0.481	0.476
Bifidobacteriales	0.314	0.069	0.297	0.225	0.503	0.326
Coriobacteriales	0.218	0.601	0.306	0.187	0.144	0.714
Bacteroidales	0.332	0.521	0.251	0.378	0.319	0.373
YS2	0.297	0.458	0.306	0.643	0.024	0.096
Streptophyta	0.652	0.859	0.715	0.133	0.280	0.185
Bacillales	0.718	0.768	0.830	0.136	0.038	0.064
Lactobacillales	0.934	0.946	0.937	0.484	0.287	0.513
Clostridiales	0.807	0.950	0.768	0.857	0.318	0.873
Erysipelotrichales	0.576	0.727	0.630	0.961	0.551	0.626
Caulobacterales	0.830	0.526	0.565	0.326	0.247	0.222
Rhizobiales	0.563	0.484	0.048	0.089	0.203	0.033
Rhodocyclales	0.892	0.260	0.306	0.081	0.387	0.049
Campylobacterales	0.176	0.250	—	0.198	—	—
Enterobacteriales	0.085	0.756	0.088	0.559	0.123	0.239
RF39	0.332	0.677	0.306	0.460	0.244	0.532
Unclassified	0.596	0.487	1.000	0.300	0.500	0.501

These results emanate from our own experiment.

Table 8.
P-value distribution of 16S rDNA gene sequence libraries compared the abundance differences of microbial communities among samples at different age period for cobb 500 broiler chicken.

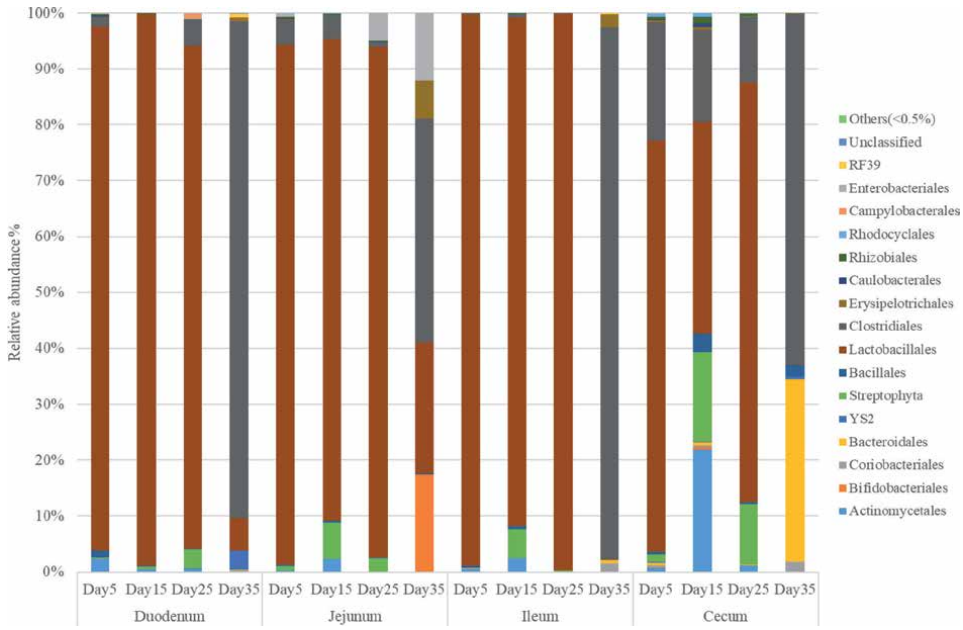

Figure 2.
Percentage of relative abundance of the bacterial community of cobb 500 broiler chicken determined from different intestinal segments at different age periods from 16S rDNA libraries. These results emanate from our own experiment.

percentage of Lactobacillales declined as the birds aged, but the cecum had a higher abundance and the percentage of Clostridiales increased as the birds aged.

4. Discussion

The luminal content samples obtained from the duodenum, jejunum, ileum, and cecum of broiler chicks at four-time points from day 5 to day 35 of age were compared in this study. Understanding how the microbiota evolves over time at specific places in the small intestine could lead to a better understanding of the chicken gut's microbial succession and dynamics. In this study, the dynamic of microbiota in the duodenum, jejunum, ileum, and cecum of broiler chickens raised in an open-sided house and supplied with a commercial diet was examined through 16S rDNA gene sequencing. The most significant finding in this work is that statistical comparisons of the compositions of distinct 16S rDNA libraries of microbial communities indicated that each region from separate intestine segments generated its own bacterial community, with very diverse relative abundances.

Our data showed that Lactobacillales were the dominant Order of bacteria in the duodenum, jejunum, and ileum through all age periods. In contrast, Clostridiales were the most abundant Order detected in the cecum at different ages. Our results agree with the previous studies [19, 20] who reported that nearly 70% of sequences from ileum were related to those of lactobacillus, whereas Clostridiaceae related sequences 65% were the most abundant group detected in the cecum. An interesting observation is that we found changes in community composition, diversity, and richness across all intestinal segments over time. More specifically, we observed an increase in the richness of microbial communities in all gut sections and a general increase in diversity.

However, the microbial community structure was moderately transient at an early age (day 5) and was replaced by a rather stable bacterial community during

the period of rapid growth, according to our findings (15–35 days of age). Other research [7, 11, 19, 20] showed similar findings, indicating that different regions of the chicken gut harbor different microorganisms and that the microbial community structure varies with age.

The microbiome is known to be affected by developmental changes in the chicken GIT as the distinct segments of the GIT become differentiated [21]. The current study discovered that the microbial composition and abundance in the four intestinal segments vary, implying that diverse intestinal microbial compositions may influence intestinal function. The alpha-diversity indices (ACE index, Chao1 index, Simpson index, and Shannon index) revealed considerable differences in microbial abundance between different intestinal portions. Gut functional variations could be caused or exacerbated by changes in microbial makeup between the four intestinal segments/locations [9, 22, 23].

5. Conclusion

Our study shows that each region of different intestinal segments developed its own bacterial community and the relative abundance was quite diverse. Further work should be directed to look in toward histological alterations related to intestinal function.

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Section 6

Nutrition

Chapter 10

Polycyclic Aromatic Hydrocarbons (PAHs) and Their Importance in Animal Nutrition

Tarkan Şahin, Sakine Dalğa and Mükremin Ölmez

Abstract

Polycyclic aromatic hydrocarbons (PAHs) formed as a result of incomplete combustion of organic compounds. It contains compounds that cause toxic, teratogenic, mutagenic and carcinogenic damage, such as heterocyclic aromatic amines, benzene and formaldehyde. PAHs can be found in industrial wastes, garbage, cigarette smoke, pesticides and flue gases and can contaminate air, water, soil and food. Although more than 100 PAH compounds are detected in nature, it is accepted that 16 PAH compounds have more harmful effects. It is important to determine the PAH exposure levels of feeds used in animal nutrition, since the contamination of feed plants and factory feeds with PAH compounds will indirectly affect human health. In this study, the physical and chemical properties of PAHs and their effects on animal production and indirectly on human health were compiled.

Keywords: benzo[a]pyrene, animal nutrition, exposure, polycyclic aromatic hydrocarbons (PAHs), feeds

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are pollutants generated by the pyrolysis or incomplete combustion of carbon-containing material, fossil fuels such as coal and other organic matter including food at high temperatures under oxygen-deficient conditions and are defined as organic lipophilic compounds that contain two or more benzene rings [1, 2]. The contamination of food with these compounds generally results from water, air and soil pollution, but PAHs may also be produced during food processing at high temperatures [3]. Owing to their chemical structure, PAHs have carcinogenic and mutagenic effects. The association of PAHs with cancer was first described in 1775 by Percivall Pott, surgeon of St. Bartholomew's Hospital in London, upon his diagnosis of scrotal cancer in chimney sweepers. This was the first observation of environmental factors being a possible cause of cancer. Later, scrotal cancer was also detected by Bell and Volkmann in workers employed in the paraffin manufacture industry in Germany and Scotland, and thereby, the observation of Pott was confirmed. In subsequent research on laboratory animals and humans, chemicals containing benzo[a]pyrene, such as tar, oil, smoke and fume, were identified as being rich in PAHs [4, 5]. Today, PAHs are produced either anthropogenically through industrialisation, the increased use of fossil fuels, waste deposition and tobacco consumption, or naturally, as a result of

forest and brush fires and volcanic eruptions [1, 6, 7]. In modern-day life, people are exposed to these compounds mostly through the consumption of contaminated water and food and the inhalation of polluted air. Exposure to PAHs increases with the consumption of heat-treated food as well as by smoking [2]. Three different mechanisms have been reported for the production of PAHs in food. The first involves the generation of PAHs as a result of the pyrolysis of organic matter such as carbohydrates, fat and protein at high temperatures (500–900°C) [8]. The second mechanism involves the generation of volatile PAHs as a result of the dripping of melting fat onto hot coal from food cooked on coal fire. These volatile PAHs contaminate the surface of cooked food at much higher levels with increased smoke generation. In particular, when meat is char-grilled, melting fat drips onto hot coal, resulting in pyrolysis, and the contact of grilled meat with smoke results in the accumulation of the volatile PAHs in the lipid components of the food. Owing to the lipophilic structure of PAHs, the water and fat content of food dictates the rate of transport of these compounds to food [2]. The third mechanism of PAH production is the incomplete combustion of coal, which eventually results in the contamination of the food surface with the generated pollutants [3].

After being discharged into the atmosphere, polycyclic aromatic hydrocarbons are either carried away from the source of emission or naturally settle in soil and water. The absorption of gaseous chemicals by plants is one of the main pathways of PAHs entering the Agri foodstuffs chain. Thus, PAHs enter the food chain of humans either directly, through the consumption of plant products such as cereals and vegetables, or indirectly, by the consumption of animal products such as milk and meat [4, 8]. The direct consumption of contaminated food and feed by livestock is the main course of organic pollutants entering the animal body. This results in animal products consumed by humans, such as meat and milk, being contaminated. Overall, 88–98% of cases of exposure to PAHs are foodborne [1, 8].

PAHs are contaminants which get in whole parts of the environment: atmosphere, waters and soils. This means that there is a risk that can be directly contaminated to plant and animal products. Considering this existence of PAHs and the risks for public health and animals associated with the exposures, the aim of this paper is to review and underline current information on the features, destinies and hazards associated with the presence of these compounds in the feeds and animal nutrition.

2. Methodology

In the present review, researches on PAHs are briefly summarised based on literatures. In this methodology, the physical and chemical properties and the most important components of PAHs are defined. For this purpose, the effects of PAHs, which have the environmental hazards, on feed and animal production are investigated. As a result, the potential risks of PAHs on human and animal health are summarised.

3. Polycyclic aromatic hydrocarbons (PAHs)

3.1 The physical and chemical properties of PAHs

Incomplete combustion does not produce a single type of PAH but results in the generation of a complex array of combustion products [5]. On the other hand, it is possible to produce PAHs as pure compounds for research purposes. PAHs,

which are produced in the form of pure compounds, are either colourless or of light yellow, white or green colour, have a light, pleasant odour and are found in solid state. Except for research purposes, these pure compounds have no further area of use [4].

PAHs, which have carcinogenic and mutagenic effects, are classified according to the number of benzene rings found in their structure. Those containing less than four benzene rings are classified as light PAHs, whilst those with more than five benzene rings are classified as heavy PAHs. Besides their classification, PAHs are also named after the number of benzene rings they possess, such that compounds with two benzene rings are referred to as naphthalene and those with three benzene rings are named as anthracene and phenanthrene. Compounds with a greater number of benzene rings have specific names [6, 9]. Light PAHs include naphthalene, acenaphthene, acenaphthalene, fluorene, anthracene and phenanthrene, whilst heavy PAHs include pyrene, fluoranthene, benzo[a]pyrene, chrysene, benzo[b] fluoranthene, indeno[1,2,3-cd]pyrene, benzo[k]fluoranthene, dibenzo[a,h] anthracene and benzo[g,h,i]perylene [4, 10]. The carcinogenicity and mutagenicity of heavy PAHs such as dibenzo[a,h]anthracene and benzo[a]pyrene are stronger than those of light PAHs [11].

The physical and chemical properties of polycyclic aromatic hydrocarbons vary with the molecular weight of the compounds [2]. Increased molecular weight is associated with reduced water solubility. PAHs with a high molecular weight are capable of enduring without evaporating, given their low solubility and volatility. Lowly volatile PAHs become even less volatile with an increase in the number of benzene rings in their structure. An increase in the molecular weight of PAHs is associated with higher boiling and melting points and lower vapour pressure. The majority of PAHs have a boiling point above 300°C and a melting point below 250°C [4, 12].

While more than a hundred PAHs have been detected in nature, among these, only 16 (**Table 1**) have been described as primary pollutants, in view of their greater toxicity and carcinogenicity [13]. This description of the primary pollutants was made by the US National Priority List (NPL) and is based on the extensive information available on these compounds, the more serious side effects, the greater residual risks, and the higher levels of detectability in hazardous dumpsite analysis [4].

3.2 PAHs in products of plant and animal origin

Environmental pollution caused by increased industrial production has resulted in the contamination of several food products, including vegetables, milk products, fruits, tea, oils, coffee, smoked meat and cereals with PAHs. Contaminated soil, water and air are known to be the main PAH contamination sources for food. Furthermore, PAHs may also contaminate food products by means of smoke generation, grilling on charcoal, processing, improper cooking methods and the use of feed additives [3, 14]. Cereals (corn, wheat, barley and oat) in industrialised regions enhance PAH levels in comparison with more outlying regions. Grain samples from a heavily industrialised region included 10 times more PAHs than samples from areas far from industry. The growth of rye near an autobahn resulted in PAH pollution, which reduced lightly 7–25 m far off from the way [15]. PAHs disrupt the growth and development of plants, which eventually reduces the overall biological activity in the ecosystem. This reduced activity also restricts productivity. Given their lipophilic nature, PAHs are deposited particularly in the double layer of membranes in plants. Plants that grow in regions with high levels of PAHs in the soil and air also contain high levels of PAHs [6, 16–18]. Thus, plants can be used to

Compounds	Chemical formula	Molecular mass (g/mol)	Toxic equivalency factor (TEF)
Naphthalene (Np)	C10H8	128.17	0.001
Phenanthrene (Phn)	C14H10	178.23	0.001
Benzo[a] pyrene (BaP)	C18H12	252	1
Fluoranthene (Flu)	C16H10	202.26	0.1
Benzo[a]anthracene (BaA)	C18H12	228	0.1
Benzo[b] fluoranthene (BbF)	C20H12	252	0.1
Indeno[1,2,3-cd] pyrene (IcdP)	C22H12	276	0.1
Benzo[e]pyrene (Bep)	C20H12	252	-
Fluorene (Flr)	C13H10	166.22	0.001
Dibenzo[a,h]anthracene (DbA)	C22H14	278	1
Pyrene (Pyr)	C16H10	202.25	0.001
Anthracene (Ant)	C17H10	178.23	0.01
Benzo[k] fluoranthene (BkF)	C20H12	252	0.01
Benzo[j]fluoranthene (BjF)	C20H12	252	-
Chrysene (ChY)	C18H12	228	0.001

Table 1.
Polycyclic aromatic hydrocarbon compounds evaluated as priority pollutants [13].

detect environmental pollution with PAHs. This also indicates that plants serve as a point of entry for PAHs into the food chain [17]. PAHs enter the human body either by the consumption of contaminated plants or by the consumption of products from animals fed on contaminated plants [1, 8].

Moreover, environmental factors may also cause the contamination of oilseeds with PAHs, such that these compounds pass into vegetable oils during the processing of oilseeds [19]. The European Food Safety Authority (EFSA) has pointed out to meat and meat products as another important source of the daily exposure of consumers to PAHs. The level of PAHs produced in meat and meat products varies with the fat content and oxygen concentration of meat, the type and temperature of the heat source used for processing, the distance maintained between the food product and heat source and the duration of processing. The direct contact of food with flames, extended heat treatment and high temperatures during processing particularly increase PAH levels [2, 10].

A previous study on the synergistic toxicity of PAHs with other pollutants investigated the combined effects of fly ash and sulphur dioxide (SO_2) on cucumbers. It was observed that neither fly ash nor sulphur dioxide showed effect alone, but when combined, the two caused severe chlorosis, which is a plant disease that manifests as the yellowing of leaves [20]. As a result, the active organic substances found in fly ash were claimed to be PAHs [17].

3.3 The effects of PAHs on human and animal health

The significant role of environmental factors in the development of cancer, one of the major diseases of the modern day, has been well acknowledged. Chemicals originating from hazardous substances, including industrial wastes, flue gases, litter, pesticides and tobacco smoke, pollute the environment, and by contaminating

air, water, soil and food, these chemicals threaten human health [4]. PAHs, including sulphur dioxide, pesticides, insecticides and nitric oxide, are carcinogenic and toxic to humans [9].

The toxicity of PAHs is not related to molecular size, but rather to the chemical structure of molecules. Generally, a carcinogenic effect is induced by the binding of PAH metabolites to deoxyribonucleic acid (DNA) [11, 12]. Once having entered the human body, PAHs cause DNA mutation. It is considered that benzo[a]anthracene and benzo[a]pyrene are particularly carcinogenic to animals and humans, respectively; thus, they are used as model compounds in cancer research [21, 22]. To exemplify, upon exposure to tobacco/cigarette smoke, benzo[a]pyrene diol epoxide adducts bind covalently to several guanine positions of the DNA p53 gene in the bronchial epithelial cells and cause cancer-inducing mutations.

Due to the potential danger posed by PAHs, food and environmental contamination risks are of high importance for human health. The fumes of fossil fuels, tobacco smoke, fruits, vegetables, bread, cereals, meat, processed and salted products and milk all contain PAHs. Moreover, the grilling or high-temperature cooking of meat and other food products increases the level of PAHs in food [4, 23]. As PAHs are generated in the form of a complex mixture of compounds, humans are most likely to be exposed to multiple PAHs at the same time. The amount of PAHs entering the human body may vary with eating, drinking, dermal contact with contaminated material and the presence of other chemical substances [24]. PAHs may enter all body tissues that contain fat. In the human body, fat is mainly deposited in the kidneys and liver. Small amounts of fat are also deposited in the spleen, ovaries and adrenal glands [4].

In order to eliminate PAHs, the human body renders them water-soluble, and this process, which involves oxidative metabolism, generates highly productive diol epoxide derivatives. These diol epoxide derivatives chemically react with DNA. Eventually, the chemical binding of PAHs to DNA causes cancer [25]. Furthermore, biological research on the placenta has shown that PAHs cause predisposition to the lung, liver, nervous system and lymphatic tissue tumours in children [3, 26]. Low IQ and childhood asthma have been reported to be associated with prenatal exposure to high levels of PAHs [27]. The Centre for Children's Environmental Health has reported that exposure to PAH pollution during pregnancy may result in adverse effects, leading to preterm labour, cardiovascular anomalies and low birth weight [12]. It is indicated that, upon PAH exposure, cancer-induced DNA damage is detected in the umbilical cord blood of babies, which may be followed by growth retardation and behavioural disorders that may increase between 6 and 8 years of age [27]. In view of these data, EFSA has stated that these compounds are potentially genotoxic and carcinogenic to humans and constitute a priority group for the assessment of health risks [12, 28].

Experimental studies on PAHs have demonstrated that animals known to have suffered from short- and long-term exposure to PAHs present with body fluid disturbances, immunity disorders and cancer of the urinary bladder, skin and lungs [4]. Following the subcutaneous and intraperitoneal injection of benzo[a]pyrene to newborn mice throughout the first 15 days after birth, it was observed that liver and lung tumours developed within a period of six months [29]. Pregnant mice exposed to very high levels of benzo[a]pyrene have been reported to display dystocia, low birth weight and other pregnancy-related problems [3, 4]. Furthermore, it is indicated that nitro-PAHs cause leukaemia and tumours of the colon and milk glands [30]. In several other research studies conducted in animals, it has been reported that exposure to PAHs, within a time frame extending from foetal development to adulthood, is highly associated with cancer development [3, 4, 26].

3.4 Previous research on PAHs

In a study conducted by Gutiérrez and Vega [1] in industrial farms located near industrial sites, the primary PAHs detected in cow's milk were reported as acenaphthene (Ace) (0.25 mg/g^{-1}), acenaphthylene (Acy) (0.32 mg/g^{-1}) and fluoranthene (Fla) (0.22 mg/g^{-1}). The most probable sources of these compounds have been suggested as contaminated grass and fuel combustion. The study reported that the milk concentrations of the 16 different PAHs detected did not exceed the dietary intake level set by the United States Environmental Protection Agency (USEPA) (25 mg/g^{-1}) and suggested that the pollutants posed a limited health risk to the animal and human populations in the study location.

Bechtel and Waldner [8] investigated the correlation between the immune functions of an annual beef cattle population, atmospheric levels of polycyclic aromatic hydrocarbons and PM_{10} oil and natural gas facilities. By analysing blood samples collected from beef cattle, the researchers determined the potential correlation between exposure to atmospheric fine particles (particles of 1 μm diameter or PM_{10}), polycyclic hydrocarbons and immune system functions. They placed herds of the annual beef cattle population at various distances to the industrial facilities located in areas producing large amounts of oil and natural gas. The researchers assessed immune system sufficiency, based on levels of B-lymphocytes and subtypes of T-lymphocytes (CD4, CD8 and WC1) in peripheral blood (n=469) and systemic antibody levels produced in response to vaccination (n=469). In this study, the mean PAH levels detected in the ambient air the animals breathed were low, and those measured at the highest levels were naphthalene (geometric mean 5.6 ng/m^3; geometric standard deviation 38) and 1-methylnaphthalene (geometric mean 2.2 ng/m^3; geometric standard deviation 12). The researchers reported not to have detected any statistically significant correlation between exposure to any of the pollutants detected in the ambient air and the immune system functions of the animals.

In another study investigating the level of exposure of dairy cattle and fattening pigs to PAHs, samples taken inside and outside the pens, in which the animals were housed, were analysed. The results of the study showed that the exposure level of the dairy cattle to PAHs was 86 times higher than the exposure level of the fattening pigs and revealed the main source of PAHs as feed for both species. The same study reported that the share of PAH intake, by means of water consumption and inhalation, in the total PAH load was negligible (**Table 2**) [31].

Samples	∑PAH		∑Cancerogenic PAH		Py		BaP		Cancerogenic PAH %	
	Cow	Pig	Cow	Pig	Cow	Pig	Cow	Pig	Cow	Pig
Indoor air (ng/m3)	56.0	25.0	4.3	1.3	7.0	3.0	0.5	0.2	7.0	5.1
Feed mixtures (μg/kg)	128.0	82.0	34.0	2.3	1.8	7.2	0.6	0.3	12.0	4.0
Water (ng/l)	38.0	100.0	11.0	51.0	2.3	8.5	0.1	6.7	8.7	9.2
Barn dust (μg/kg)	4475.0	676.0	162.0	38.0	33.6	26.0	1.2	1.1	17.0	9.0

Py: average pyrene concentration; BaP: average benzo(a) pyrene concentration (% carcinogenic. PAH).

Table 2.
PAHs contamination from cows and pigs barns [31].

In their research investigating the presence of PAHs in the tissues and internal organs of pigs and cattle, Ciganek and Neca [32] analysed specimens taken from the liver, lungs, kidneys, eyeballs (lens and vitreous body), muscles and fat tissue. Analyses revealed the presence of PAHs in the following internal organs and tissues of pigs and cattle at the indicated levels in ng/g, respectively: liver (3.8, 2.7), lungs (4.6, 5.4), kidneys (5.4, 6.3), fat tissue (0.05, 0.11), muscle tissue (3.6, 5.1), lens (57.9, 16.3) and vitreous body (14, 6.4). The most common PAHs detected in the specimens were phenanthrene, pyrene, naphthalene and fluorenone. As a result, in this study, no statistically significant difference was observed between the PAH levels detected in the organ and tissue samples of the pigs and cattle. However, the PAH levels detected in the tissue samples of animals of the same species housed in the same pen were found to significantly differ.

In the studies carried out to reduce the contamination of PAHs in the soil, there are also results that alfafa, ryegrass and Juncus subsecundus plants reduce the concentration of pyrene and phenanthrene compounds in the soil [33–35].

4. Conclusion

In conclusion, the production of feedstuff, both naturally and from industrial by-products, for use in animal husbandry, poses the risk of exposure to PAHs. This risk points out to the need for conducting PAH analyses in feedstuff used for the production of animal products, which have an important place in the food chain of humans. It is evident that the number of available studies on the impact of PAHs on the environment and animal health is rather limited [12, 18, 36]. In this context, it is considered that determining the impact of PAHs on human nutrition and health by fully demonstrating the adverse effects of PAHs on animal nutrition and health, as well as developing remediation strategies, is of utmost importance.

Conflict of interest

The authors declare no conflict of interest.

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This volume presents selected issues in the complex and diverse science of animal husbandry. The use of computer programs provides an opportunity to improve breeding and optimize farm management. At the same time, the use of traditional breeding methods is also of decisive importance. Knowledge of animal welfare and animal wellness is of great help in controlling animal health issues and in economic production. In the biological processes of reproduction of dairy cows, the events of the 100 days after calving are of fundamental importance. Production systems influence the process of product production, in which the relationship between animal products and human health goes far beyond animal husbandry, and to which the issue of greenhouse gases is also connected. The quality of manufactured meat products is influenced by both on-farm and off-farm factors, but good meat cannot be produced from low-quality animals, even with excellent slaughterhouse work. Background knowledge of animal health □ including the microbiome in the digestive tract, which makes use of the feed □ makes this activity more effective, which is of particular importance in the case of broiler chickens. Knowing the behavioural characteristics of animals (rams) enables better management. Many horse breeds are capable of artificial gaits as a result of breeding and selection processes. Comparative knowledge of the movements of these horse breeds also helps to understand their differences. The quality of life of animals and the quality of manufactured products are also affected by polycyclic aromatic hydrocarbons from the environment, which, being stored and enriched in fat-containing tissues, can also have adverse effects on the human consumer. Each topic presented not only offers specialist knowledge but makes interesting reading in its own right.

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