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ENERGY AND PROTEIN STANDARDS FOR FINISHING BELGIAN BLUE DOUBLE-MUSCLED BULLS

ENERGIE- EN EIWITNORMEN VOOR BELGISCH WIT-BLAUWE DIKBILSTIEREN IN DE AFMESTFASE

door

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Gontrode, mei 2000

De promotoren: *De auteur:*

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Sam

MaPa,
bedankt
…

mits wat zeuren werd 26 juli mijn dag
of is het toch die van jullie (glimlach)

sindsdien, alsof het geluk het zo had gepland
maar eigenlijk, gestuurd door jullie hand
ben ik in dromenland
beland

tevreden
restte er me heden
jullie nog te overreden

van de kracht
die mij tot dat geluk heeft gebracht

sam

'Some people are so ignorant of animal economy as to imagine that it is to no purpose to feed cows when they are giving no milk or horses when not at work. I met some years ago with Rev. Clergyman, attending a funeral of one of his parishioners, mounted on a large meagre horse and labouring hard with a staff and spur to keep up with the procession. When requested to accompany the funeral to the place of interment, he said, he found his horse unable to travel which, he said, was to him surprising, as he had given him no less than three measures of grain that morning. The clergyman owned, that he had given the horse no grain since last he rode him, about three weeks previous. Some farmers seem to have adopted the notion of this Rev. Gentleman as to their dairy cows.'

William Aiton, «View of the Agriculture of

Ayrshire» 1811.

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List of abbreviations

adjR^2	Adjusted R^2
BB	Belgian Blue
BIA	Bioelectrical impedance analysis
BRE	Undegraded feed crude protein
CC	Right carcass half
CF	Crude fibre
CP	Crude protein
CT	Computerised tomography
CV	Coefficient of variation
DCF	Digestible CF
DCP	Digestible CP
DEE	Digestible EE
DEn	Digestible energy
dm	Double-muscled
DM	Dry matter
DNFE	Digestible NFE
DP	Decreasing protein group
DPIE	Decreasing protein and increasing energy group
DS	Deuterium space
DVBE	Digestible undegraded feed protein
DVE	True protein digested in the small intestine
DVEc	DVE corrected for a negative OEB
DVME	Microbial protein digestible in the small intestine
DVMFE	Endogenous protein losses in digestion
DXA	Dual-energy X-ray absorptiometry
E	Energy
EB	Empty body
EBA	Empty body ash
EBEn	Empty body energy
EBF	Empty body fat
EBP	Empty body protein
EBW	Empty body weight
EBWa	Empty body water
EE	Ether extract
EMME	Electronic meat measuring equipment
EMS	Electromagnetic scans
fLW	Fasted live weight
FOS	Fermentable organic matter
GEn	Gross energy
IE	Increasing energy group
L, M or H	Low, moderate or high E or P

LBM	Lean body mass
LT	*m. longissimus thoracis*
LW	Live weight
$LW^{0.75}$	Metabolic LW
MEn	Metabolisable energy
MREE	Microbial protein based on available energy
MREN	Microbial protein based on available nitrogen
MRI	Magnetic resonance imaging (=NMR)
N	Number
n.r.	Not reported
nc	Non double-muscled
NC	Negative control group
NCP	Non carcass parts
NEF	Net energy for fattening (MJ)
NEn	Net energy
NFE	N-free extract
NIRS	Near infrared reflectance spectroscopy
NMR	Nuclear magnetic resonance (=MRI)
ODS	Undigested dry matter
OEB	Degraded protein balance
P	Protein
PUN	Plasma urea-nitrogen
r	Pearson correlation coefficient
R^2	Determination coefficient
RSD	Residual standard deviation
SD	Standard deviation
SU	Sugars
T0, T12, T18, T24	0, 12, 18 and 24 minutes post mean infusion time
UCE	Urinary creatinine excretion
US	Urea space
US12, US18, US24	Urea space at T12, T18 and T24
VEVI	Feed unit beef cattle intensive (1 VEVI = 6.908 kJ)
VIA	Video image analysis
Y	Year

I General introduction, aim and framework of the thesis

I.1 General introduction

I.1.1 Origin of the Belgian Blue breed

The Belgian Blue breed originates from 'Het ras van Midden en Hoog België' (the breed of Middle and High Belgium). At the end of the 19th century, the geographical position of Belgium as the centre of numerous commercial exchanges between different European countries, had markedly determined the cattle population in that region (Anonymous, 1991b). The population was a mixture of different dairy breeds with some Shorthorn and Durham blood. Until 1890, official directives for breeders were lacking and the crossing with different breeds continued, resulting in a population with interesting characteristics concerning milk production, external conformation and early maturation. In the beginning of the 20th century, the first official directives resulted in a dual-purpose breed in which milk production was still predominant. Since then, milk production has lost of its importance within the breed and from 1960-1970 on a general tendency towards extreme conformation is noted (Hanset, 1982). In that period the first attempts to commercialise the breed on international manifestations indicated the necessity to change the name of the breed to 'Het Wit-blauw ras van België' (the Belgian Blue breed). By that time, in some animals the meatiness was so predominant that the milk production had decreased drastically. Therefore in 1974, the breed was divided in two sub-populations: the extreme meat type and the dual purpose type (Anonymous, 1991b). Now, 26 years later, the first sub-population largely dominates the breed and is characterised by an extreme musculature and conformation (due to the double-muscling phenomenon) which surpasses that of any other breed in the world. The phenomenal extent of the muscularity has made the Belgian Blue breed world-famous for research on metabolism and development and for the mapping of the double-muscling gene.

I.1.2 Importance of the Belgian Blue double-muscled bulls for meat production in Belgium

Within the Belgian cattle industry, the importance of the Belgian Blue (BB) double-muscled (dm) bulls has markedly increased during the past two decades. This is a consequence of the continuous expansion of the suckler cow herd: in 1980, 137239 suckler cows accounted for 12 % of the total cow population in Belgium whereas in 1998 that number had more than tripled, being 493132 or 44 % (NIS, 1999a). The main shift from dairy cattle towards suckler cows was caused by the introduction of the milk quota system in 1984. The surplus of roughage and stables that became available by the reduction of the dairy herd, was mainly used for suckler cows and fattening bulls. This shift was reinforced by the European agricultural policy that awarded the farmers with premiums for suckler cows and fattening bulls.

Figure I.1.1 demonstrates the evolution of the proportion of the different cattle categories within the total number of slaughtered animals from 1963 to 1998 (NIS, 1999b). The total number of slaughtered veal calves has decreased with about 10 %

between 1963 (346106) and 1998 (311083). The lowest number of calves was slaughtered in 1973 (228065). Whereas in 1963 the number of slaughtered cows (249213) clearly exceeded the number of bulls (178632), in 1998 this difference decreased towards less than 3000 units (275701 cows and 272735 bulls). These figures prove the drastic increase in the importance of bulls for meat production. In 1963, 16 % of the slaughtered animals were bulls, while in 1998 that figure had increased towards 30 %. This was mainly possible thanks to a drastic decrease in the number of slaughtered steers (108179 in 1963 *vs*. 11923 in 1998). The last category has become really marginal since only 1.3 % of the slaughtered animals in 1998 were steers. This is a consequence of the change towards more intensive beef production systems in Belgium during the last decades. On the one hand, that change had become unavoidable due to the limited surface for agricultural production. On the other hand, the dm animals of the BB breed are well fit for intensive production systems.

No direct figures seem to be available on the percentage of beef that is produced in Belgium from BB dm bulls. However, the importance of the breed within the total cattle population in Belgium has undeniably increased during the past 20 years. Whereas in 1980 only 36 % of all cows belonged to the BB breed, in 1998, that figure has increased towards more than 52 % (NIS, 1999a). According to Hanset (1996), 80 to 85 % of the BB population was double-muscled at that time. That percentage is expected to have increased ever since.

Because of the unique geretic potential of these dm BB animals and the intensive production systems in our country, Belgium produces the best carcasses in Europe. In 1993, 60.9 % of the carcasses of bulls slaughtered in Belgium were classified as 'S' or 'E' (according to the European SEUROP-system; Anonymous, 1991a). In France and Spain (2nd and 3rd in Europe) only 15.5 and 10.5 % of the carcasses respectively, received an 'S' or 'E' score (Bouquiaux and Hellemans, 1996). Two years later, the importance of the double-muscling phenomenon seemed even more important, as 72.3 % of the slaughtered bulls were given the 'S' or 'E' score. For that year, in France and Spain, the figures decreased towards 10.4 and 10.2 % respectively (Bouquiaux, 1998; personal communication).

The figures of Bouquiaux and Hellemans (1996) (72.3 % of the bulls slaughtered as 'S' or 'E') give the best indications that the percentage of beef produced by the BB dm bulls is predominant in Belgium. The same authors estimated that in 1994, 75 % of the red meat produced in Belgium originated from cattle belonging to the BB breed.

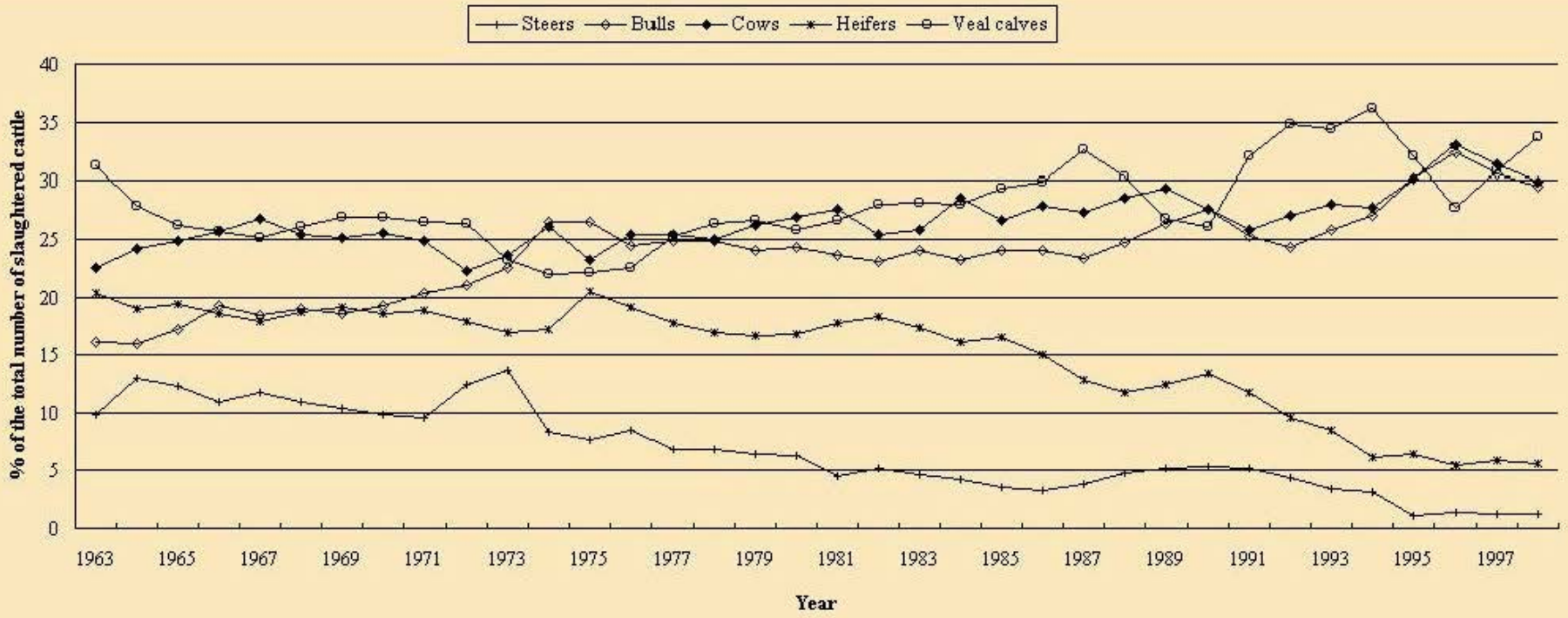

Figure I.1.1: Evolution of the proportional partition of the total number of slaughtered cattle over different categories (NIS, 1999b)

I.3 Aim of the study

Efficient beef production with the BB dm bulls is only possible if adequate rations are fed. Until recently no energy or protein standards existed for that type of animal. Farmers tended to feed excess of protein, to avoid any shortage that could cause reduced performances. An excessive protein content in the ration has important disadvantages. It is economically unjustifiable, the animal has to spend extra energy to excrete the surplus of protein via the urine and the increased N-excretion is an unnecessary load for the environment. So, facing an increased economical and ecological interest, the farmer has to produce more efficiently, taking the environment into account. Besides, optimising energy and protein might also improve slaughter quality, which is important for the financial revenue of the farmer. Therefore, the need for energy and protein standards for BB dm bulls had become more and more urgent.

With this study we aimed to resolve that need by deriving the optimal energy and protein content in the ration for optimal growth while taking the carcass and meat quality in consideration. Since the body composition changes with increasing live weight and consequently the need for protein and energy, we aimed to adjust protein and energy in the ration to the differing needs in the course of the fattening period. This was realised by determining energy and protein standards for BB dm bulls during the fattening period (350 - 650 kg), for each live weight category of 50 kg, and for different growth rates within each live weight category. The energy and protein standards will be expressed in VEVI and DVE respectively, as these are the current units for energy and protein evaluation used in Belgium.

I.4 Framework of the thesis

During the past decades lots of research has been done on the double-muscling phenomenon. From the results, it is clear that the conformation as well as many other characteristics of the dm animal is deviant from those of non double-muscled (nc) animals. In **Chapter II.1** a short review is given to describe the most important characteristics of the double-muscling phenomenon and to illustrate that the BB dm bull is so deviant from any other breed that research determining separate energy and protein standards for these animals is really justified.

As mentioned before, the standards had to be expressed in VEVI and DVE, according to the energy and protein evaluation systems currently used in Belgium and The Netherlands. The VEVI-system was first introduced in The Netherlands from 1977 onwards, as the new energy evaluation system replacing the starch-equivalent system. Later on the DVE/OEB-system replaced the VRE-system. In **Chapter II.2** both systems will be discussed shortly.

The framework of this thesis is shown in Figure I.3.1. A large dataset was derived from two feeding trials, conducted during three and two years respectively and involving a total of 333 BB dm bulls. The first feeding trial studied the influence of six

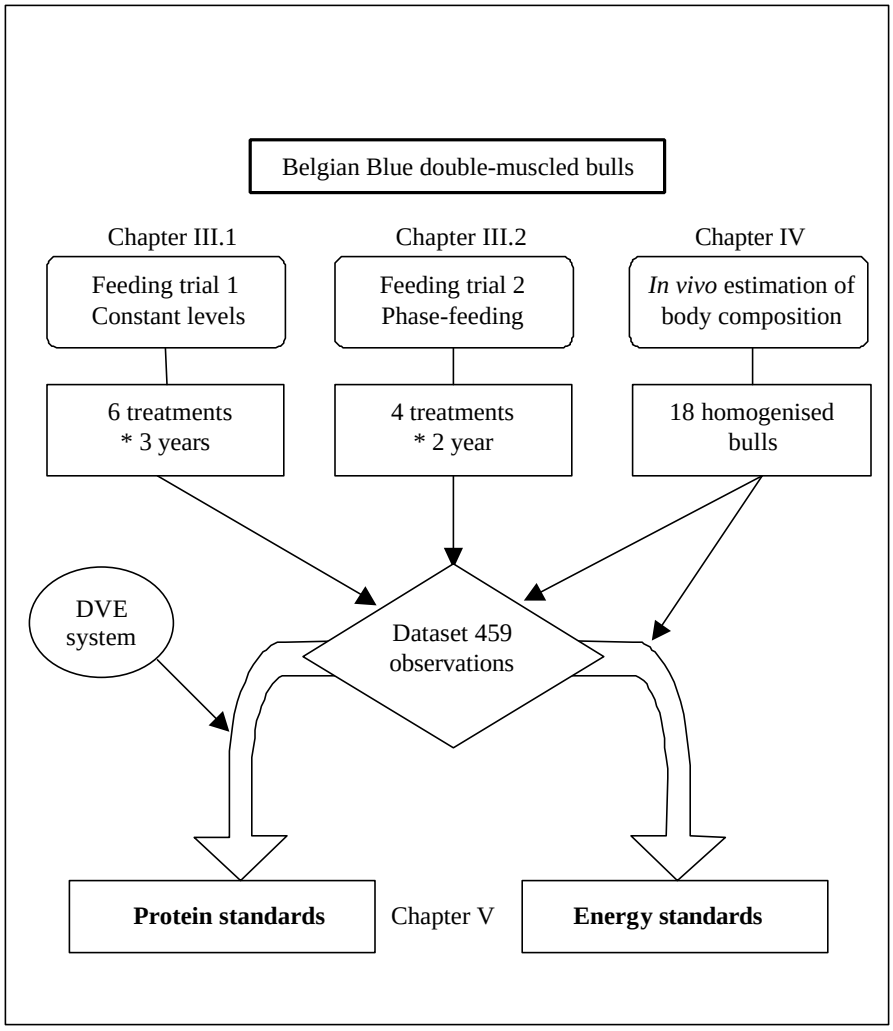

Figure I.3.1: Framework of the study

different energy-protein combinations on the performance and on the carcass and meat quality of BB dm bulls.

Therefore, two energy levels, and within each energy level, three protein levels were fed during the entire finishing period. The treatments of that trial are described and the results are reported in **Chapter III.1**. Based on the results of that first trial, a second trial was designed. The influence of phased energy and protein feeding on

the performance and carcass quality was investigated. The trial combined four different energy-protein treatments. The aim was to adjust the protein and energy feeding to the changing needs of the animals, with increasing live weight. This second feeding trial is described and its results are reported in **Chapter III.2**.

To determine energy and protein standards, data about body composition are as important as the data of the feeding trials. Although the dm bulls have been the subject of many research projects, the actual body composition of these animals was never determined. In general, an estimation of the tissue composition (meat, fat and bone) was made, but chemical composition was never determined. In this study, a total of 46 bulls were used during two consecutive years to evaluate two different techniques to estimate body composition *in vivo*. The choice of which techniques would be evaluated was done based on an extensive literature review regarding the most important techniques for *in vivo* estimation of body composition in cattle. At that time, taking the facilities of our research institute into account, urea infusion and urinary creatinine excretion (UCE) were the two techniques that were retained. The review on the *in vivo* estimation techniques has lately been updated and that latest version is to be found in **Chapter II.3**.

In **Chapter IV.1** both retained techniques for the *in vivo* estimation of body composition will be described, as well as the results of their application. Shortly after applying both techniques, 18 of the 46 bulls involved in this study were slaughtered and subsequently homogenised to determine chemical body composition. Combining the results of the homogenisations and the prediction techniques allowed us to determine equations estimating the body composition of the bulls. From a comparison of the different equations, the best estimation technique was selected, to be used in the final determination of the changes in the body composition. As mentioned before, these data are essential for deriving the standards.

More detailed results on the body composition of the 18 dm bulls are to be found in **Chapter IV.2**. In that chapter the *in vivo* estimation technique that was withheld in Chapter IV.1 is used to determine the protein and energy accretion over different live weight ranges.

Based on the results of the feeding trials (Chapter III) and the compositional data (Chapter IV.2), a dataset was derived. In **Chapter V** explanation will be given on how the energy - and protein standards were calculated from the dataset. Finally, in that same chapter, the energy and protein standards will be given and discussed.

Chapter II

Literature review

General outline of Chapter II

A first question arising from reading the title of this thesis will probably be if separate standards for Belgian Blue double-muscled bulls are necessary. During the past decades lots of research has been done on the double-muscling phenomenon. The results have indicated that the conformation as well as many other characteristics of the dm animal is deviant from non double-muscled animals. In **Chapter II.1:** "**Why separate standards for Belgian Blue double-muscled bulls**?" a short review is given to describe the most important characteristics of the double-muscling phenomenon and to illustrate that the BB dm bull is so deviant from any other breed that research determining separate energy and protein standards for these animals is really justified.

The energy and protein evaluation systems currently used in Belgium and The Netherlands are the VEVI- and the DVE-system. In **Chapter II.2: "The current protein and energy systems in Belgium: DVE and VEVI"** both systems will be discussed shortly and an overview will be given of the different systems used in other countries.

To be able to quantify the compositional changes of Belgian Blue double-muscled bulls, a separate trial was designed to evaluate techniques, used for *in vivo* estimation of body composition. Preliminary, literature concerning most *in vivo* estimation techniques for ruminants was reviewed and from that review two techniques were selected, taking the facilities of our Department into account. That review has lately been updated and is given in **Chapter II.3: "*In vivo* estimation of body composition in cattle"**.

II Literature review

II.1 Why separate standards for Belgian Blue double-muscled bulls?

II.1 Why separate standards for Belgian Blue double-muscled bulls?

In this chapter, it will be demonstrated that BB dm bulls are really different from other bulls, and that separate energy and protein standards are justified. In the first part, a short review will be given on the characteristics of a dm animal and on the general consequences of the double-muscling phenomenon. In the second part, these characteristics that might influence energy and protein requirements are highlighted.

II.1.1 Double-muscling phenomenon

Introduction

Different countries or languages have different popular terms for the phenomenon of muscular hypertrophy. In Great-Britain and the USA the animals demonstrating the hypertrophy are called double-muscled, in Italy 'a groppa dopia' (double rumped), in Germany 'doppellender' (double loin), in France 'cul de poulain' or 'culard' and in the Dutch speaking countries they are called 'dikbillen'. Although the name double-muscled is not really correct (a duplication of the muscles is never found), that term will be mostly used throughout this work as it is most common.

In the beginning of the 19th century the first references were made on the muscular hypertrophy. Cully described the phenomenon in 1807 (cited by Oliver and Cartwright, 1968). According to him, the animals showing the phenomenon originated from the "Shorthorn" cattle developed in England, after importation from The Netherlands.

Nowadays, different breeds demonstrate the double-muscling phenomenon. Vissac (1982) listed 11 European breeds in which the hypertrophy was found, with the main populations being: Piemontese, Charolais and Belgian Blue. Oliver and Cartwright (1968) concluded that the condition likely originated with the cattle native to the Low Countries of Western Europe. It probably was introduced into many of the important present day breeds of beef cattle from the Shorthorn breed. Other cattle of European origin likely received the genes for the phenomenon through the use of cattle from the Low Countries in improving native cattle.

Genotype

The tremendous variety of expression of the double-muscling phenomenon has led to different theories concerning the inheritance of the characteristic. Boccard (1981) concluded that most geneticists believed that the phenomenon is based on a recessive gene with incomplete penetrativeness. According to Arthur (1995), different theories concerning the inheritance have been proposed by several authors, varying from a recessive to a dominant character. Hanset and Michaux (1985a and b) postulated for the BB bulls the existence of an autosomal *mh* (muscular hypertrophy) locus characterised by a wild type "+" allele and a recessive "*mh*" allele, causing the dm phenotype in the homozygous condition. Heterozygous "*mh*/+" animals, although phenotypically closer to "+/+" animals, exhibited some degree of muscular hypertrophy, which has led the

authors to refer to the *mh* allele as being partially recessive. Charlier *et al.* (1995) added that the gene truly deserved to be qualified as a major gene since the difference between the heterozygous and the homozygous animals amounts to four standard deviations for some measures of muscularity. The same authors located the *mh* gene on the bovine chromosome 2. Somewhat later Casas *et al.* (1998) refined the location and it was finally Grobet *et al.* (1997) who determined with BB bulls that the characteristic was caused by a deletion of 11 basepares in the bovine myostatin gene. Bass *et al.* (1999) explained that the expression of myostatin, as a muscle growth inhibitor by limiting fibre number and to some extent fibre size, is highest in bovine muscle during gestation when muscle fibres are forming. The latter summarised that a shortage of functional myostatin is associated with an increase in fibre number. That results in a marked increase in potential muscle mass in dm cattle.

Phenotype

Although the double-muscling phenomenon is well known, it is still difficult to define it unambiguously. This is mainly a consequence of the large variation in the expression of the gene. Some phenotypic characteristics however are very common in dm animals. The most obvious external phenotypic characteristics, were summarised from Oliver and Cartwright (1968) and Kieffer and Cartwright (1980):

- muscular hypertrophy of the hindquarter
- muscular hypertrophy of the shoulders
- enlarged *m. longissimus dorsi*
- legs wide apart
- smaller and lighter head
- shorter and thicker neck
- short tail with setting more forward
- thin skin in combination with very few subcutaneous fat
- creases between the muscles may be seen through the skin
- fineness of the bones

Most of the above mentioned characteristics have a positive influence on the performances of the animals, however other characteristics are less positive (summarised from Oliver and Cartwright (1968) and Kieffer and Cartwright (1980)):

- underdeveloped external genitalia
- hocks are extremely straight or in some cases crooked
- calves have a hypertrophied tongue
- calves may show bucked-over or bowed-out front legs

Most problems associated with the newborn calves disappear after a few weeks. However, during the first weeks calves with a hypertrophied tongue may have serious problems with suckling, especially since teats of a dm cow are generally enlarged (Arthur *et al.*, 1989a) and less well-formed than those of dairy cattle. At the same time, the milk and colostrum production of the dm cows does not always meet the requirements of the calf, since milk production seems to be reduced (Vissac *et al.*,

1974). Based on the results of different authors, Ménissier (1982) concluded that milk production is decreased with 15 to 30 % in double-muscled cows.

The hypertrophy of the calf in combination with a reduced width of the pelvic inlet (Hanset *et al*., 1989) can cause birth difficulties. Within the current BB population about 95 % of the calves are born with caesarean. This has been criticised more and more over the past years, especially by the northern European Countries, for reasons of animal welfare. Others indicate that a caesarean has become more and more common practice and that it could even prevent (if a specialist does it) complications and stress that can occur during natural birth (Bergström and Oostendorp, 1985; Vermorel *et al*. 1989). Due to the caesareans, the incidence of perinatal mortality is very low in the BB breed (Hanset, 1982). Clauwers *et al*. (1999) observed a calf loss of 1.1 % within 15 days after birth, which is considerably lower than in most other cases (Bellows and Short, 1994).

Besides the high frequency of caesareans, other problems may occur concerning reproduction. A reduced fertility was mentioned by Oliver and Cartwright (1968) originating from poor sexual behaviour, genital infantilism, delay in puberty and reduced sexual drive (Michaux and Hanset, 1981; Ménissier, 1982 and Arthur *et al*. 1989a). These symptoms are present as well in male as in female cattle (Ménissier, 1982).

Performance and carcass characteristics

In general, double-muscled bulls have proven to combine a high growth rate with an improved feed conversion compared to nc bulls. The improved feed conversion is caused by a lower feed intake capacity (Geay *et al*., 1982), due to a reduction in the size of the digestive tract (Ansay and Hanset, 1979; Boccard, 1981). Fiems *et al*. (1995a) offered 75 dm BB bulls, within a 375 to 620 kg live weight range, a diet consisting of 50 % maize silage and 50 % concentrates. They found a mean daily gain of 1.44 kg and a DM intake of 5.82 kg per kg gain. Minet *et al*. (1996) gathered results of 16 trials with 129 dm BB bulls fed on concentrate diets. They found an average growth rate of 1.47 kg per day and a DM conversion of 6.13, over a mean range of 330 to 576 kg. Clinquart *et al*. (1991) found with 60 BB dm bulls between 375 and 575 kg a mean daily gain of 1.70 kg. These bulls were fed *ad libitum* a concentrate diet based on sugar beet pulp, cereals, soya-bean meal, linseed meal and middlings.

Apart from these excellent performances, the main economic advantage of the muscular hypertrophy is the extreme conformation of the carcass. A special class 'S' was added to the European Classification scheme for bovine carcasses (Anonymous, 1991a), because the carcasses of the BB dm bulls clearly excelled the originally highest class: the 'E' class.

Different trials have compared animals with normal conformation and dm ones. They all confirmed that dm animals have a higher dressing percentage, a reduced fat content and fat covering, a high proportion of muscle within the carcass and an improved conformation (Ansay and Hanset, 1979; Boccard, 1981; Michaux *et al*., 1984; Arthur *et al*., 1989b; Clinquart *et al*., 1994; Uytterhaegen *et al*., 1994; Fiems *et al*., 1995c). Bouton *et al*. (1978) found reduced but still important differences between heterozygous dm animals and homozygous non double-muscled (nc) animals. In Table

II.1.1 and Table II.1.2 results of two comparisons between BB dm and nc bulls are given.

Both tables clearly illustrate the double effect of the muscular hypertrophy on the quantity of meat that is produced by one animal. First, the dressing proportion is importantly increased, meaning that at a comparable live weight, the carcass weight is increased. Secondly, the carcass contains a higher proportion of muscles in dm animals. For example, a dm animal weighing 650 kg contains 343 kg meat, while a nc animal of the same live weight only contains 272 kg meat (according to the results of Table II.1.1).

Table II.1.1: Comparison of some carcass characteristics between BB dm and nc bulls according to Fiems et al. *(1995c)*

Characteristic	Double-muscled	Normal conformation	Pooled SD[†]
N	108	185	
Live weight (kg)	695[a]	651[b]	37
Carcass weight (kg)	483[a]	419[b]	29
Dressing proportion (%)	69.5[a]	64.3[b]	1.4
SEUROP Conformation[‡]	17.4[a]	12.0[b]	1.4
SEUROP Fat covering[§]	4.6[a]	7.9[b]	1.3
Carcass composition (%)			
Meat	75.9[a]	65.0[b]	2.5
Fat	11.3[a]	21.7[b]	2.7
Bone	12.8[$]	13.3[$]	n.r.

[a,b]: means in a row with different superscripts are significantly different (P < 0.001)
[†] Standard deviation
[‡] S = 18, E = 15, U = 12, ..., P = 3 points
[§] Class 1 = 3 (very lean), Class 2 = 6, ..., Class 5 = 15 points (very fat)
[$] calculated by difference

Table II.1.2: Comparison of some carcass characteristics (mean (SD[†])) between BB dm and nc bulls according to Uytterhaegen et al. *(1994)*

Characteristic	Double-muscled	Normal conformation
N	32	59
Live weight (kg)	693[a] (38)	608[b] (34)
Carcass weight (kg)	484[a] (28)	378[b] (24)
Dressing proportion (%)	69.9[a] (0.9)	61.7[b] (1.3)
SEUROP Conformation[‡]	17.0[a] (1.1)	10.2[b] (1.5)
SEUROP Fat covering[‡]	4.5[a] (1.0)	8.7[b] (1.1)
Carcass composition (%)		
Meat	75.9[a] (1.6)	63.1[b] (2.5)
Fat	11.2[a] (1.6)	23.0[b] (2.7)
Bone	13.0[a] (1.0)	13.9[b] (1.0)

[a,b]: means in a row with different superscripts are significantly different (P < 0.001)
[†] Standard deviation
[‡] see Table II.1.1

When comparing carcasses of dm and nc animals with the same carcass weight, apart from a few exceptions (*e.g. m. diafragmaticus*) all muscles within the carcass of the dm animal will be heavier then in the carcass of the nc animal. This would indicate a general hypertrophy of the muscles. However, Boccard and Dumont (1974) compared dm and nc animals with the same muscle mass and found muscles showing a hypertrophy and consequently others showing a hypotrophy. It was concluded from this and other studies (*e.g.* Hanset *et al.*, 1982; Shahin and Berg, 1985b), that the hypertrophy was most pronounced in superficial muscles *(m. cutaneus trunci* +34 % and *m. latissimus dorsi* +17 %), while the deep muscles showed a hypotrophy (*m. vastus medialis* -38 % and *m. obliquus internus abdominis* -19 %). The muscles that are in connection with the respiratory system were also less developed in dm animals (*e.g. m. diafragmaticus* -18 %).

The hypertrophy is not caused by an increase in the section of the muscle fibres, but basically from a hyperplasy of the muscle fibres (West, 1974). This multiplication of the cells takes place during the foetal phase (Boccard, 1981), resulting in dm cattle possessing nearly twice the number of muscle fibres at birth as normal cattle (Gerrard *et al*., 1991). The number of fibres in a section of a muscle is also increased by the lengthening of the fibres (Boccard, 1981). Batjoens *et al.* (1991) found a decrease in the cross-sectional area of fibre type I, IIA and IIB in the *m. longissimus thoracis* (LT) in dm bulls compared to nc ones, but only the first two differences were significant.

Although the increased muscle mass is mostly mentioned as the most prominent characteristic of the dm phenomenon, discriminant analyses have indicated that the hypodevelopment of the fatty tissues is more important in characterising the syndrome (Dumont, 1982; Shahin and Berg, 1985a). The reduced fatty tissues are the result of a reduction in the volume of the fat cells rather than a decrease in fat cell numbers (Bailey *et al.*, 1982).

Apart from the increased volume of the muscle mass, the strongly reduced size of the fifth quarter is also responsible for the increase in dressing percentage. Based on different studies Boccard (1981) quoted different authors finding an important hypotrophy of most organs. Ansay and Hanset (1979) compared dm and nc calves at the same weight (83 kg) and found an important reduction of the different parts of the fifth quarter in dm animals. The skin, thymus, spleen, lungs, liver, kidneys, digestive tract and heart showed a reduction of 19.8, 51.1, 37.3, 19.4, 17.2, 19.0, 17.2 and 14.9 % respectively. Geay *et al.* (1982) calculated that the digestive tract counts for 5.3 % of the total live weight in normal animals, whereas for dm animals this is only 4.6 %.

Meat quality

Besides the higher dressing proportion of dm animals, we already mentioned that dm animals also have a higher percentage of carcass lean meat. But even more important, there is the added advantage that more primal cuts are found within the total muscle mass (Hanset *et al.*, 1989, Arthur, 1995). In Table II.1.3 and Table II.1.4 a comparison of meat quality characteristics between Belgian Blue dm and nc animals is made.

*Table II.1.3: Comparison of some meat quality characteristics (*m. longissimus thoracis*) between BB dm and nc bulls according to Fiems* et al. *(1995c)*

Characteristic	Double-muscled	Normal conformation	Pooled SD
N	108	185	
pH_{24}	5.6[a]	5.6[b]	0.1
Cielab L*	39.4[a]	38.5[b]	2.6
Shear force (N)	50.7[a]	44.2[b]	11.0
Water holding capacity (cm^2)	6.5	4.7	0.7
Meat composition (%)			
Moisture	75.7[a]	74.1[b]	1.0
Fat	0.9[a]	3.2[b]	1.0
Protein	23.0[a]	22.6[b]	0.6

[a,b]: means in a row with different superscripts are significantly different ($P < 0.05$)

*Table II.1.4: Comparison of some meat quality characteristics (*m. longissimus thoracis*) (mean (SD)) between BB dm and nc bulls according to Uytterhaegen* et al. *(1994)*

Characteristic	Double-muscled	Normal conformation
N	32	59
pH_{24}	5.5[a] (0.1)	5.5[b] (0.1)
Shear force (N)	59.8[a] (14.1)	38.3[b] (9.3)
Sarcomere length (µm)	1.80 (0.16)	1.74 (0.10)
Cooking losses (%)	30.0[a] (2.3)	25.6[b] (2.8)
Drip losses (%)	7.2[a] (1.5)	5.4[b] (2.6)
Collagen (% of DM)	1.61[a] (0.16)	2.5[b] (0.42)

[a,b]: means in a row with different superscripts are significantly different ($P < 0.001$)

In recent years, meat quality has become a major research topic. As meat tenderness is the most important organoleptic parameter determining meat quality (Dransfield *et al.*, 1982; Koohmaraie, 1992), lots of comparisons have been made of meat tenderness between dm and nc animals. However, seemingly contradictory results were found. Arthur (1995) reviewed the literature and concluded that most of the recent reports indicated that meat from dm cattle is more tender, provided that the meat has been correctly chilled and aged. Boccard (1981) and Bouton *et al.* (1982) and several others attribute the improved tenderness to a lower collagen content, which is generally found in dm animals (Bailey *et al.*, 1982; Hanset *et al.*, 1982). Bailey *et al.* (1982) indicated that the collagen content is not only lower, but it is made up of a lower proportion of stable non-reducible cross-links. However, Fiems *et al.* (1995c), Uytterhaegen *et al.* (1994) (Table II.1.3, II.1.4) and Clinquart *et al.* (1994) found an increased shear force value without any indication of an inappropriate chilling and ageing process. Uytterhaegen *et al.* (1994) indicated that in the BB dm animals, collagen content is indeed strongly decreased but the decrease is largely compensated for by a decreased postmortal myofibrillar tenderisation, resulting in tougher meat. Seemingly, in the BB breed the dm phenomenon is so pronounced that postmortal myofibrillar degradation is reduced. This has a negative influence on high quality muscles whose tenderness is mainly determined by the postmortal tenderisation since

the collagen content of these high quality muscles is low. In low quality muscles, containing more collagen, the final tenderness is mainly determined by that collagen content, and therefore, the reduction in collagen content of dm animals can improve final tenderness of these muscles. Demeyer *et al.* (1995) concluded that cooking conditions are probably predominant when comparing the tenderness of meat from dm and nc animals, since heating the meat to a temperature of 70°C largely removes the difference between both types in the collagenous component of tenderness. This was confirmed by De Smet *et al.* (1998) who found that tenderness of meat from dm animals may be underestimated by heating samples up to 75°C in comparison with raw meat. Accordingly, De Smet *et al.* (1999) found with raw meat of BB bulls lower shear force values in higher graded carcasses (with the dm bulls being graded highest), while shear force values of cooked meat did not vary much across the SEUROP grading scheme.

Table II.1.3 illustrates another important characteristic of the meat of dm animals. The intramuscular fat content has decreased below 1 %. As fat is found to be responsible for cardiovascular diseases, a reduction of fat is of interest for reasons of public health (Reckless, 1987; Klurfeld, 1994).

Not only the composition of the muscle, but also the occurrence of the different fibre types differs between the two types of cattle (Table II.1.5). Fiems *et al.* (1995c) found in accordance with West (1974), Batjoens *et al.* (1991) and Stavaux *et al.* (1994) significantly more white and less red fibres in dm animals. As the white fibres are glycolitic and the red ones are oxidative or oxidative-glycolitic, dm animals shift towards a more glycolitic metabolism in the muscle.

Table II.1.5: Comparison of fibre type occurrence in m. longissimus thoracis *(mean % (SD)) between dm (N = 4) and nc (N = 7) bulls according to Fiems* et al. *(1995c)*

Fibre type	Double-muscled	Normal conformation	Significance
I (red)	29.0 (5.4)	32.0 (2.9)	
IIA (red)	22.1 (3.4)	31.0 (7.0)	P<0.01
IIB (white)	48.9 (4.8)	37.0 (6.4)	P<0.01

Besides the shift in fibre type and area, the muscles of dm animals also have a lower capillary density. This was mentioned by Ashmore and Robinson (1969) and Stavaux *et al.* (1994). Although the lower capillary density might influence the colour of the muscle to some extent, two other factors are mainly responsible for the paler colour that is generally found (Bouton *et al.*, 1982; Fiems *et al.*, 1995c) in dm animals: the earlier mentioned shift towards more white muscle fibres and a lower concentration of myoglobin (Bailey *et al.*, 1982; Ouhayoun, 1982; Fiems *et al.*, 1995c). According to Purchas (1991) both factors might be correlated since type I fibres contain more myoglobin. This was earlier suggested by Morita *et al.* (1970) who indicated that myoglobin concentration is greater in red muscle than in white.

II.1.2 Consequences of the dm phenomenon on the protein and energy requirements

Some of the anatomical differences between dm and nc animals may have important effects on the physiology and as such on the nutritional requirements of these animals. Ménissier (1982) already stated in 1982 that dm animals have specific nutritional requirements that are probably different from those of normal animals, but have generally not yet been established.

The reduction of the digestive tract (Ansay and Hanset, 1979; Geay *et al.*, 1982) can result in a reduction of the daily intake or in a higher passage rate. The latter can reduce digestibility. In both cases the uptake of nutrients is decreased, which should be compensated for by an increased nutrient concentration in the diets. Different studies have meanwhile indicated that the intake of dm animals is reduced in comparison with nc animals (Holmes and Robinson, 1970; Hanset *et al.*, 1979; Clinquart *et al.*, 1995; Fiems *et al.*, 1997 and 1999b). Fiems *et al.* (1997) could only find a significantly increased CP digestibility for dm bulls compared to nc bulls, while DM, CF, EE, NFE and energy digestibility were comparable. The difference in CP digestibility disappeared when DM intake was used as a covariant. These results mainly confirmed earlier findings of Holmes and Robinson (1970) and Vermorel *et al.* (1976 and 1994) who did not found any influence of the double-muscling phenomenon on the DM digestibility.

Different comparisons of carcass compositional data indicated that dm animals demonstrate a large decrease in percentage fatty tissue, and at the same time an increase in muscle content. This would indicate that dm animals have higher protein needs, unless they have a better protein efficiency or a lower protein turnover. Van Eenaeme *et al.* (1989 and 1991) found indications for a 'slower' protein metabolism due to a lower synthesis, degradation and consequently a lower net accretion. This 'slower' protein metabolism however does not completely compensate for the extra protein deposition, as Boucqué *et al.* (1984) concluded from a feeding trial that BB dm bulls have higher protein needs than BB nc bulls.

Opposite indications concerning the energy requirements make it much more difficult to determine whether dm animals have higher or lower energy requirements. As was mentioned for protein, the energy content of the diet should be higher for dm bulls, if the energy requirements are comparable, because the daily intake is lower (Fiems *et al.*, 1999b) and no improved energy digestibility was found (Fiems *et al.*, 1997). The lower fat content in the dm animals suggests a lower need of net energy for deposition. However, Geay (1984) calculated that efficiency of utilisation of metabolisable energy (MEn) decreases from 0.6 towards 0.3 if the proportion of total retained energy that is retained as protein increases from 0.1 to 0.5. This could indicate that the efficiency of energy utilisation is very low for dm animals, as the protein deposition seems to dominate fat deposition during the total fattening period.

Pipes *et al.* (1963) found indications for a reduction of the energetic maintenance requirements in beef cattle in comparison with dairy cattle. The authors explained this difference by a reduced activity of the thyroid gland. Hanset *et al.* (1987) calculated reduced maintenance requirements for BB dm bulls in comparison with BB nc bulls (131 vs. 142 kcal metabolisable energy/kg $LW^{0.75}$). Vermorel *et al.* (1976) found no difference in energy requirements for maintenance between dm and nc Charolais bulls. However, the requirements for those animals were 12 % lower than for Friesian bulls. Geay *et al.* (1982) indicated that dm animals show lower activity and have a slower protein turnover due to a less developed digestive tract and to a higher proportion of white fibres. It is well known that the digestive tract has the highest cellular turnover of the whole body. Taylor *et al.* (1986) found the maintenance requirements for dairy cattle to be 20 % greater than that of beef breeds. However, Russel and Wright (1983) concluded that the maintenance requirements are positively related with the total protein mass, meaning that thinner animals with the same weight have higher requirements than fatter ones. This is caused by the difference in the respective maintenance cost of protein and fat. Ferrell and Jenkins (1984a, b en 1985) believe that differences in weights of the vital organs, that are metabolically very active, may explain most of the differences found. Vermorel *et al.* (1994) found for a comparable energy intake, that BB dm calves had 11 % higher lying energy expenditures than BB nc calves, mainly due to the increased muscle mass. The general concept is that MEn requirement for maintenance tends to be lower per unit $LW^{0.75}$ in fat animals than in lean animals (Dickerson, 1985; Webster, 1985).

The high dressing proportion as shown in Table II.1.1 and II.1.2 were partly due to a decrease in the fifth quarter. From the butcher point of view this is very positive, but the reduction of some organs has an important influence on the sensitivity to acute respiratory diseases (Michaux *et al.*, 1984) and on the susceptibility to stress.

Halipré (1973) diagnosed an increased susceptibility to thermal stress. When environmental temperature increases, the large muscle mass of dm animals causes larger heat production and their body temperature increases more rapidly than in normal cattle. The lowered possibilities for heat dissipation (reduced respiratory capacity), their lower exchange surface per mass unit and their lower blood circulation are the main reasons for that increased susceptibility. Campbell (1988) stated that animals of higher genetic potential for lean tissue growth are more sensitive to nutritional stress than those of lower lean tissue growth potential. Holmes *et al.* (1973) concluded from two trials that dm animals are easier excitable as nc ones. When blood was taken using a catheter, dm calves had somewhat lower lactic acid blood values than nc, but when blood was taken by puncture, the blood lactic acid value of the dm animals was higher. Holmes and Ashmore (1972) suggested that the greater excitability might enhance their susceptibility to stress. Holmes *et al.* (1973) also subjected dm en nc animals to three kinds of stress: nutritional, psychic and exercise stress. In the three cases they found evidence that the dm animals were more susceptible to stress. Lekeux and Van De Weerdt (1996) concluded that dm BB calves had a reduced capacity to withstand physical exercise in comparison with Friesian calves.

The higher susceptibility to stress clearly can have important repercussions on protein and energy requirements, since stress can alter the steady state of the body and challenge complex physiological adaptive processes (NRC, 1996). The absolute amount

of energy needed for that reaction is dependent on the amount and duration of the excitation. However no absolute value can be given concerning the energy expenditure for stress. In case of heat stress, NRC (1984) suggested that the respiration of the animal gives an indication of the severity of heat stress. For rapid shallow breathing, maintenance energy requirements should be raised with 7 %, whereas for deep open-mouth panting requirements should be increased up to 25 %.

The drastic anatomical, physiological and biochemical changes and the favourable performances, all caused by the double-muscling phenomenon, may have important repercussions on the energy and protein requirements. Concerning the protein, it is quite obvious that higher requirements may be expected, while for the energy it is much more difficult to make a prediction. Some factors, such as the very low fat content and the presumed lower maintenance requirements, suggest lower energy requirements. The higher stress susceptibility suggests higher requirements.

II Literature review

II.2 The current protein and energy systems in Belgium: DVE and VEVI

II.2 The current protein and energy systems in Belgium: DVE and VEVI

II.2.1 Introduction

During the past decades productivity of dairy and beef cattle has increased drastically. This is mainly due to a continued progress in the genetics and a better understanding of ruminant nutrition. The advance in the ruminant nutrition science resulted in new feed evaluation systems in The Netherlands and Belgium. Concerning the energy evaluation, in the early eighties, the VEVI-system replaced the 'starch equivalent' (van Es, 1978; van Vliet *et al.*, 1994). Later on, in 1991, a new protein evaluation system replaced the Digestible Crude Protein (VRE; Verteerbaar ruw eiwit) system. The new system was called the DVE/OEB system: true protein digestible in the intestine/degraded protein balance (DarmVerteerbaar Eiwit/Onbestendige Eiwit-Balans) (Tamminga *et al.*, 1994). Both systems are currently in use in Belgium and The Netherlands. Before explaining the basics of both evaluation systems, a short description will be given on the protein and energy metabolism in ruminants.

II.2.2 Protein metabolism in ruminants

Degradation of the feed protein starts in the rumen with a partial breakdown to peptides, amino acids and ammonia. The amount of protein that is broken down depends on the type of ration, the type of protein, the feeding level and the passage rate. The ammonia, amino acids and peptides from the protein breakdown are used by the microbes to produce microbial protein. Provided that N is not limiting and that the essential growth factors are present and available in the rumen fluid, the energy supply is the most determining factor in the intensity of the microbial protein synthesis.

Ammonia that is not used, diffuses through the ruminal wall into the blood and is transformed to urea by the liver. The urea is then excreted by the urine or recycled to the saliva, the rumen or to the hindgut.

The protein arriving in the duodenum is a mixture of undegraded feed protein, microbial protein and endogenous protein, with the first two being most important since these provide the (essential) amino acids. The amino acids absorbed from the duodenum are used for maintenance (protein turnover, energy source...) and production (milk, wool, and protein accretion). The efficiency of the use of the amino acids is different for each of these purposes. The quality of the protein present in the duodenum depends on the similarity between the amino acid composition of that protein and the composition of the needs.

Finally, a microbial fermentation takes place in the hindgut, which only has a minor importance for the protein metabolism, since ammonia is the sole protein product that is absorbed in the hindgut.

II.2.3 DVE/OEB-system

As mentioned before, the VRE-system was replaced by the DVE/OEB-system. The VRE-system was only based on the difference between CP (crude protein; N * 6.25) ingested with the ration and CP excreted in the faeces. The current protein evaluation system is an important improvement of the previous system because it accounts for the important changes that the protein goes through in the rumen. The system is based on two important parameters: DVE and OEB. The calculations of these parameters are schematically represented in Figure II.2.1.

That scheme clearly indicates that DVE is the total of the digestible undegraded feed protein (DVBE) and the microbial protein digestible in the small intestine (DVME) minus the endogenous protein losses in digestion (DVMFE).

DVE = DVBE + DVME - DVMFE

The first term is dependent on the fraction of undegraded feed CP in total feed CP (% BRE) and on the digestion in the small intestine of the undegraded feed protein (% DVBE).

DVBE = CP * 1.11 % * % BRE * % DVBE

The second term is determined from the fermentable organic matter (FOS), which is a measure for the total amount of energy available for the microbes in the rumen. FOS is calculated from the digestible organic matter minus the nutrients that are not available for the micro-organisms in the rumen.

DVME = FOS * 0.150 * 0.75 * 0.85

The different coefficients in the latter equation concern the efficiency and digestibility. It is assumed that for each kg FOS, 150 g microbial protein can be formed, that 75 % of the microbial protein are amino acids and that 85 % of these amino acids are digestible in the intestine.

The third term is a measure for the amount of protein that is lost due to the digestive process. It is estimated from the indigested dry matter (ODS), assuming that for each kg ODS 75 g DVE is needed for endogenous protein losses.

DVMFE = ODS * 0.075

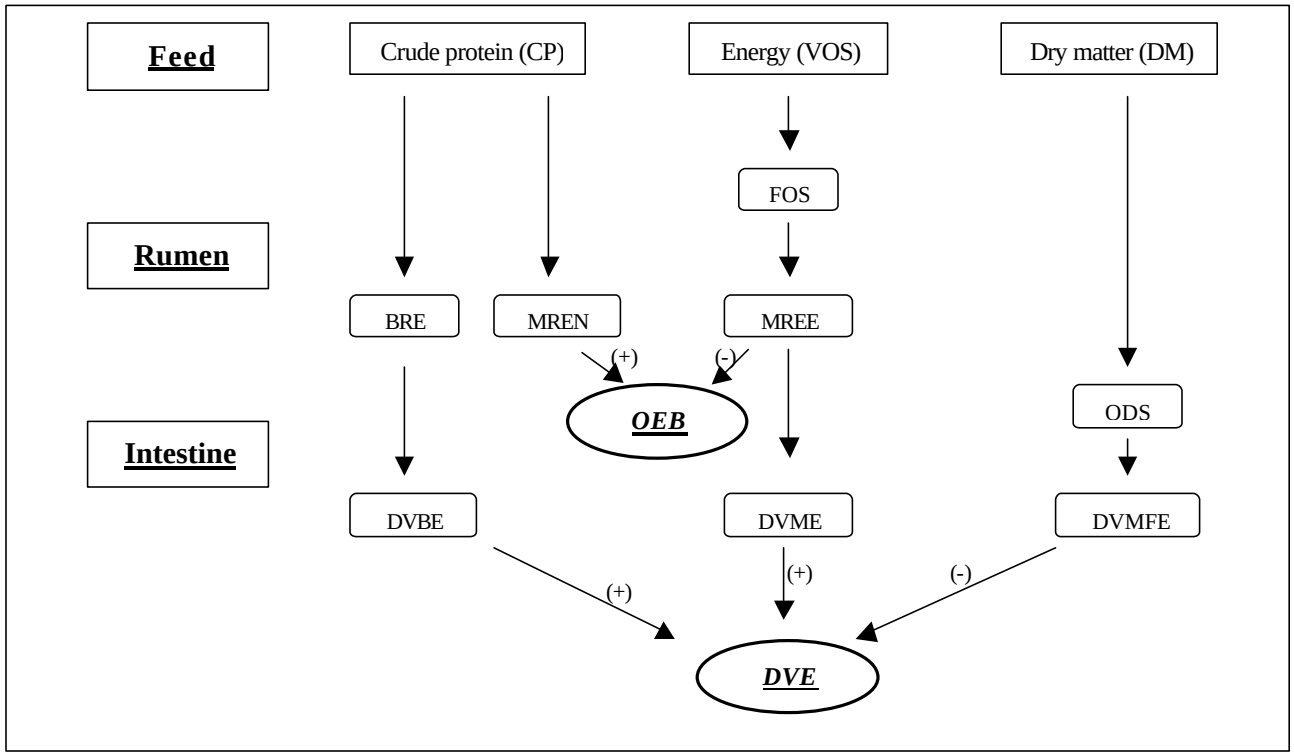

Figure II.2.1: Overview of the different steps in the calculations of the DVE/OEB evaluation system

From the last three equations, DVE can be calculated, but that value is only valuable if the OEB (degraded protein balance) is positive. The OEB is calculated as the difference between the amount of microbial protein that can be formed based on available nitrogen (MREN) and the amount of microbial protein that can be formed based on available energy (MREE).

OEB = MREN - MREE

with MREN = CP * [1-(1.11*% BRE)]

and MREE = FOS * 0.150.

II.2.4 Protein evaluation systems in use in different countries

Although the different systems that are currently in use in different countries have different names, the principle of all these protein evaluation systems is comparable (Table II.2.1). They all estimate the amount of amino acid nitrogen that reaches the duodenum, the amount of it that is digested and consecutively used for maintenance or production. However several differences exist between the systems concerning the calculation and the quantification of the amount of digestible protein. These differences have been discussed in detail by several authors (Jarrige and Alderman, 1987; Andries *et al.*, 1989; AFRC, 1992).

Table II.2.1: Overview of some protein evaluation systems in use in different countries

Country	Name of the system	Reference
Belgium The Netherlands	DVE/OEB	Tamminga *et al*. (1994) van Vliet *et al.* (1994)
UK	Rumen degradable protein (RDP) Undegraded protein (UDP)	ARC (1984)
	Metabolisable protein (MP)	AFRC (1992)
France	Protein digested in the intestine (PDI)	INRA (1978) Vérité and Peyraud (1988)
Italy	adapted from PDI	Susmel and Piva (1987)
Switzerland	Absorbable protein in the intestine (API) (from PDI)	Bickel and Landis (1987)
Nordic countries (Denmark, Norway, Sweden, Finland, Iceland)	Amino acids truly absorbed in the small intestine (AAT) - Protein balance in the rumen (PBV)	Madsen (1987) Madsen *et al.* (1995)
USA	Metabolisable protein (AP)	NRC (1996)
Australia	Apparently digested protein leaving the stomach (ADPLS)	Corbett *et al.* (1987)

The most important difference between the DVE/OEB system and most other systems is the calculation of DVMFE. Within the DVE-system that parameter is considered to be dependent on the characteristics of the feedstuff, and consequently it is a part of the feed evaluation. All other systems consider the faecal loss of N as a part of the maintenance requirements. As such, comparison of the protein standards expressed in DVE with the others systems is very difficult.

II.2.5 Energy metabolism in ruminants

All animals derive their energy from degradation of organic compounds. The utilisation of ingested feed energy by animals involves several kinds of losses (van Es and Boekholt, 1987). Only part of the nutrients can be digested, the remainder is excreted in the faeces and is lost for the animal. The difference between the gross energy value of the feed and that of the associated faeces is the apparent digestible energy (DEn) (Blaxter, 1962). A further loss of energy occurs in the form of gases, mainly methane (Demeyer and Van Nevel, 1975). This loss is particularly important in ruminants (ADAS, 1984). Apart from the gasses, the urinary losses, containing organic waste products, also contain energy that is of no further use for the animal. The difference between the DEn and the gaseous and urinary losses is termed the metabolisable energy (MEn). All that energy can be utilised by the organism. However, not all of the MEn is of use for the animal since animals continuously produce heat, when fasting, when masticating, when ruminating, when digesting…, even the micro-organisms in the rumen cause a further heat loss (van Es and Boekholt, 1987). Subtracting the total heat losses from the MEn gives the net energy (NEn), which is that part of the gross energy that is used by the animal for maintenance or production. Schematically, Table II.2.2 (from Buysse *et al.*, 1977 and Shirley, 1986) shows the consecutive losses of a feedstuff during digestion.

According to Vermorel (1988) the major part of the absorbed energy are the volatile fatty acids (between 64 and 75 % of the total absorbed energy), while the amino acids can deliver 15 to 28 %. The long chain fatty acids represent 5 to 15 % while glucose is only representing 1 to 4 % of the total absorbed energy.

Table II.2.2: Overview of the different energy losses during digestion

Energy	Influenced by
Gross-energy (GEn)	
↓ - faecal losses	• ration characteristics • feeding level • physical structure of the ration • type of animal
Digestible energy (DEn)	
↓ - urinary losses - gaseous losses	• ration characteristics • feeding level • age of the animal • type of animal
Metabolisable energy (MEn)	
↓ - thermal losses	• ration characteristics • physiological function • type of animal
Net energy (NEn) for maintenance for production	

II.2.6 VEVI-system

The name of the energy evaluation system currently used in Belgium and in The Netherlands, stands for 'Voedereenheid Vleesvee Intensief' (Feed unit beef cattle intensive). The system evaluates the NEn value of the feedstuffs, *i.e.* the amount of energy the animal actually disposes off for its maintenance and for production. This NEn is only a fraction of the gross energy (GEn).

Within the new energy system, a quantity of 6908 kJ has been equated with 1000 VEVI. This corresponds with the mean NEn content of one kg barley that is disposable for an intensively fed bull. As such 1 VEVI corresponds with 1 g barley, being 6.9 kJ. Although the theoretical background for calculating the net energy value is quite simple, the practical calculations are somewhat more complex. Therefore not all details concerning the calculations will be given. More details on the VEVI system are published by van Es *et al.* (1978) and van Vliet *et al.* (1994).

1. The first step is to calculate the GEn and the MEn of the feedstuff from the following equations.

$$GEn = 24.14*CP + 36.61*EE + 20.92*CF + 16.99*NFE - 0.62*SU$$

with GEn = gross energy expressed in kJ/kg DM; CP = crude protein, EE = ether extract, CF = crude fibre, NFE = N-free extract and SU = sugars, all expressed in kg per kg DM.

$$MEn = 15.90*DCP + 37.66*DEE + 13.81*DCF + 14.64*DNFE - 0.62*SU$$

with MEn = metabolisable energy expressed in kJ/kg DM; DCP = digestible CP, DEE = digestible EE, DCF = digestible CF, DNFE = digestible NFE and SU = sugars, all expressed in kg per kg DM.

For the calculation of the different parameters in the second equation, digestibility coefficients are available in literature (*e.g.* CVB, 1999).

2. The second step is to calculate the proportion of MEn within the GEn, being called the factor 'q'.

$$q = MEn/GEn *100$$

3. The third and final step calculates how much of the MEn remains as NEn. This is calculated by multiplying MEn with k_{mf}. In the same equations, the transformation from kJ towards VEVI is done (factor 6.908 = the energy value of 1 kg barley in MJ). The factor k_{mf} is dependent on the proportion of k_m (efficiency for maintenance) and k_f (efficiency for fattening) and the APL (animal production level).

$$VEVI = k_{mf} * MEn/6.908$$

$$VEVI = \frac{k_f}{\frac{k_f - k_m}{APL * k_m} + 1} * \frac{MEn}{6.908}$$

with k_m and k_f both being dependent on the factor q.

II.2.7 Energy evaluation systems in use in different countries

Two kinds of energy evaluation systems are in use in different countries. On the one hand the systems based on net energy and on the other hand the systems based on metabolisable energy. From the systems mentioned in Table II.2.3, only the British system is based on MEn. The first three systems are strongly comparable, and only differ with regard to some details. As such the Dutch and French systems have introduced the feed unit (neither calorie nor Joule), while the Swiss system uses the Joule as unit. Whereas the feed unit in the French system corresponds with 7.615 kJ, in the Dutch system one VEVI (for beef cattle) or VEM (for dairy cattle) equals 6.908 kJ.

All the results in this study were first calculated as VEVI, but were later on converted to Joule since that is the international accepted unit. These converted figures will be referred to as NEF (net energy for fattening).

Table II.2.3: Overview of some energy evaluation systems in use in different countries

Country	Energy standards	Reference
Belgium The Netherlands	VEVI/VEM	van Es (1978) van Vliet *et al*. (1994)
France	UFV/UFL	Vermorel (1978) Vermorel (1988)
Switzerland	NEW/NEL	Bickel and Landis (1978)
Britain	MEn	ADAS (1984)
Scandinavia	SFU	Flatt *et al*. (1972)
USA	NEn	NRC (1984)

II Literature review

II.3 *In vivo* estimation of body composition in cattle

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II.3 *In vivo* estimation of body composition in cattle

II.3.1 Introduction

The composition of an animal is influenced by a lot of factors. Most important are the genetic origin of the animal (*e.g.* breed, meatiness), the sex, the weight and external factors such as quality and quantity of feed. Table II.3.1 shows some examples of the influence of each of these factors on the composition of the carcass or empty body. The most relevant factor seems to be the conformation. A double-muscled (dm) Belgian Blue (BB) bull has on average, depending on the source, 10.9 (Fiems *et al.*, 1995c) or 12.5 percent units (Clinquart *et al.*, 1994) more muscle in the carcass than a BB nc bull. In addition, the fat percentage is respectively 10.4 and 12.6 units lower according to the same authors. The carcasses of breeds that are used for meat production *ipso facto* contain more muscle than the carcasses of dairy breeds. Clinquart *et al.* (1994) showed that dm BB bulls have 17.1 percent units more muscle in their carcass than Holstein bulls, being a relative difference of 30 %. The fat content on the contrary is more than 50 % lower. Shahin (1995) found in a comparison of dm animals that bulls had 4.9 percent units more muscle and 5.5 percent units less fat than their female equivalents. The influence of body weight (Robelin and Daenicke, 1980) can be seen as a shift towards more lipids and less protein with increasing live weight. The influence of feeding on composition is more complex. Fiems *et al.* (1999a) for instance found an effect of the level and degradability of starch in the concentrates on the fat content of the carcass. The influence of additives, *e.g.* hormones, on body or carcass composition has been reported by numerous authors (Shackelford *et al.*, 1992; Boucqué *et al.*, 1994; Moloney *et al.*, 1994; Vestergaard *et al.*, 1994; Fiems *et al.*, 1995b).

For scientists a good estimation of body composition is very important in order to analyse the continuous changes in the animal's body composition during the experimental period without an expensive serial slaughtering procedure. Based on these changes the needs for nutrients during the successive growth stages can be determined and the effect of feeding below or above requirements on the final body composition can be investigated. A good estimation technique could also be used to divide the experimental animals in homogeneous subgroups at the beginning of an experiment. Furthermore, a good estimation technique to predict animal composition is a basic tool, in optimising the amount of meat by genetic or nutritional manipulation of the composition of an animal.

Because accuracy is much more important for the scientist than the speed of measurement, research has concentrated on more complex techniques rather than on simpler methods suitable for on-farm use, although the latter are commercially very important.

Table II.3.1: Comparison of some factors influencing the composition of cattle

Carcass composition estimation from 3 rib-cut		% Muscle	% Fat	% Bone
Breed (Clinquart *et al.*, 1994)	Holstein bulls	57.6	25.2	17.2
	BB nc† bulls	61.2	24.2	14.4
	BB dm† bulls	74.7	11.6	13.7
Carcass composition estimation from 1 rib-cut		**% Muscle**	**% Fat**	**% Bone**
Meatiness, type (Fiems *et al.*, 1995c)	BB nc bulls	65.0	21.7	13.3
	BB dm bulls	75.9	11.3	12.8
Carcass composition from one side dissection		**% Muscle**	**% Fat**	**% Bone**
Sex (Shahin, 1995)	Male dm	74.3	14.0	10.9
	Female dm	69.4	19.5	10.4
Empty body composition from total analysis		**% Water**	**% Lipids**	**% Protein**
Weight (Robelin and Daenicke, 1980)	Charolais bulls 163 kg	71.5	5.6	18.7
	Charolais bulls 724 kg	64.0	13.2	18.9
Carcass composition estimation from 1 rib-cut		**% Muscle**	**% Fat**	**% Bone**
Feeding (Fiems *et al.*, 1999a)	High starch (nc bulls)	68.6	18.4	13.0
	Low starch (nc bulls)	65.5	21.8	12.7
Additives (Boucqué *et al.*, 1994)	Control group (dm bulls)	75.2	12.2	12.6
	Cimaterol group (dm bulls)	79.4	8.2	12.4

† nc = non double-muscled / dm = double-muscled

The most frequently mentioned reasons to estimate the composition of an animal's body in practice are:

- to enable producers to meet the consumer's demand for a leaner product (*e.g.* by selection towards leaner animals based on body composition estimation)
- it could be the basis of a value-based marketing system (Cross and Whittaker, 1992) because the value of a meat-producing animal depends greatly on its composition
- it could help the farmer in selecting those animals of his stock that are ready for slaughter
- it enables the producer to provide adequate amounts of nutrients according to the requirements of the animal.

Scientists, consumers, livestock producers and the meat industry all have interest in an accurate prediction technique to estimate the body composition of animals. In order to know the composition of an animal, the most precise technique is the quantitative analysis of the whole body. This is relatively simple for small animals like mice, rats, chickens and even piglets. But in the case of cattle, it is not only very laborious, but also very expensive and time-consuming. For total analysis, one should collect all parts of the fifth quarter, during the slaughtering procedure, and afterwards homogenise and analyse them. To analyse the carcass part, only one half of it should be homogenised. From the composition of both parts total body composition can be calculated. It is obvious that this technique is not acceptable for daily practice. Therefore, a good non-destructive technique to estimate body composition is needed, because it can save time, energy and resources.

Different techniques exist to estimate body or carcass composition post mortem: prediction of the composition of the carcass from the dissection of a sample joint (Torreele and Verbeke, 1966; Ledger *et al.*, 1973) or from characteristics of a one rib-cut (Verbeke and Van de Voorde, 1978; De Campeneere *et al.*, 1999b); prediction according to the tissue-sawdust technique (Williams *et al.*, 1974) based on the analysis of the sawdust collected from sawing through the frozen carcass or parts of it on specified places, *e.g.* through the frozen round, loin, rib and chuck at 2.54 cm intervals (Topel and Kauffman, 1988); prediction based on carcass measurements (Kempster, 1986); prediction from the total fatty tissue weight (Robelin and Geay, 1978), prediction from the composition of the non-carcass parts (De Campeneere *et al.*, 1999c), prediction based on NIRS (Near Infrared Reflectance Spectroscopy) (Mitchell *et al.*, 1996) etc.

Because simple extrapolation of the composition data from slaughtered animals towards a random weight is not possible, due to the changing composition of an animal during its growth, *in vivo* estimation of body or carcass composition is needed, to know the composition of an animal that can not be slaughtered. For example when we are interested in the change in composition over a certain time-interval or due to a certain treatment.

The research for reliable techniques to estimate the body or carcass composition of an animal *in vivo* resulted in different methods. Some of them require expensive equipment, some take too much time to be practicable in industry, others don't give a satisfying estimate. Despite all these problems many researchers are still

looking for a cheap, fast and practically applicable technique to predict body composition in live animals, which proves that it is urgently needed, for scientific as well as for industrial purposes. The main difference between these two application fields is that for the industry the price and the convenience of use are more important, whereas for scientific purposes the accuracy and precision prevail. For the consumer and the meat industry the carcass composition is predominant. For the nutritionist however, an animal needs nutrients not only for producing its carcass but also for the other parts of the body and for its maintenance (van Es, 1981).

The different kinds of techniques for *in vivo* estimation of body composition are: on the one hand the infusion techniques with deuterium, tritiated water or urea and on the other hand the external measurements of characteristics of the animal with all kinds of equipment: starting from the simple weight of the animal, over linear or volumetric measurement towards more complicated techniques such as conductivity or impedance measurements.

Although this review concerns *in vivo* prediction of body composition in cattle, some other techniques will also be dealt with and comparisons will be made with other species.

II.3.2 Estimation techniques

Linear measurement and subjective evaluation

The most simple and less expensive technique to estimate the composition of an animal is based on the evaluation of the external conformation. It is undoubtedly the most commonly used technique in practice. It can be based either on linear measurements or on a subjective evaluation or visual assessment. The first method consists of numerous measurements of length, height, circumference and other linear measurements, using all kinds of specially elaborated callipers, on the non-symmetrical surfaces of the animal. Based on different publications, Topel and Kauffman (1988) and Kempster (1986) decided that linear measurements are not useful as an individual predictor for the muscle, fat and bone content of animals. The only easily obtainable measurement on live animals that can have some predictive value is the body weight. O'Mara *et al.* (1998) found a correlation r = 0.55 between live weight and total fat percentage. Wright and Russel (1984a) on the contrary found strong relations (R^2 = 0.912 and R^2 = 0.918) between live weight (LW) and respectively body fat and body protein with mature cows of five breeds. Velazco *et al.* (1997) published equations, for Holstein steers between 3 and 12 months of age, predicting kg water and protein in the carcass with an R^2 = 0.97 and 0.96 respectively. With mixed-breed steers (210 to 517 kg), Hammond *et al.* (1988), found an R^2-value of 0.93 between empty body weight (EBW) and kg protein in the empty body (EB). The same researchers found for the same parameters but with Angus steers (219 – 517 kg) a R^2 = 0.87. Reid and Robb (1971) mentioned a very high correlation between the same parameters: R^2 = 0.997. However, these results were obtained with heifers ranging from 1 day to 14 months (± 45 to 240 kg). Wilkinson and Greenhalgh (1995) found R^2-values of 0.942 and 0.967

between EBW and kg protein and water in the EB respectively, with 66 lambs ranging in live weight between 14 and 58 kg.

In the subjective evaluation the animals are scored for several diverse characteristics, *e.g.* frame size, body capacity, health and fleshing score (Strasia *et al.*, 1989) or condition, capacity, muscle, frame and quality score (Smith *et al.*, 1989). A common problem for the evaluator is to distinguish between fatness and muscling. Visual assessments are more reliable as indicators of muscling within a narrow range of fatness, particularly when the level of fatness is low (Kempster, 1982). On the other hand, visual appraisal is very subjective, meaning that scores for the same animal may vary with the evaluator. The results of Gresham *et al.* (1986), also with mature cows, indicated that carcass composition could be predicted from both objective and subjective measurements, with the accuracy and precision needed for many purposes. Gregory *et al.* (1998) found a relation with a r = 0.75 between condition score and % body fat with 40 Friesian and Friesian-cross cows. Rémond *et al.* (1988) found a r = 0.88 between condition score and kg fat in the body. Wright and Russel (1984b) also stressed the importance of condition scoring to predict body composition and particularly body fat, for different purposes because the technique is very simple, inexpensive and fast. In agreement with the above mentioned authors, Lewis *et al.* (1969) found that the estimation made by trained evaluators accounted for, on the average, over twice the variation of the estimation made by untrained personnel.

The palpation technique is the most frequently used technique to estimate the quantity of subcutaneous fat (Agabriel *et al.*, 1986) and consequently to evaluate if the animal is ready to be slaughtered. Several spots exist to palpate the animal, with the most important ones being the flank, the tailbone, the udder- or scrotum, the breast (between the legs and behind) and the last rib. Although this technique is very commonly used in practice, it is of little scientific value.

The linear measurement and subjective evaluation techniques are very fast and often based on the experience of the operator. In practice, this technique is although not really satisfying, usually the only alternative. When this technique is used for experiments, it is very important that the operator is well trained and that the same operator evaluates the animals during the complete trial.

Video image analysis (VIA)

To avoid subjectivity influencing the visual assessment, techniques were developed to measure conformation and body size unbiased. Kallweit (1982) mentioned photogrammetry to evaluate and estimate the volume of the animals or certain fractions of it. With two pairs of synchronised flash-light-equipped cameras, one pair placed behind and one pair alongside the animal at well-defined distances, total volume or fractions of the volume were estimated using a computation program.

VIA is based on a camera/computer system and could be considered as a more developed stage of the photogrammetry. It can also be seen as a replacement for or a supplement to the visual assessment, but the VIA-technique is not biased by subjectivity as is the visual assessment, and is not dependent on the operator. This technique is mostly applied on carcasses to evaluate conformation, but can also be used to predict *in vivo* body composition (Sørensen, 1983; Cross and Whittaker, 1992).

Wassenberg *et al.* (1986) found a coefficient of determination of 95.6, using the VIA on cross sections of carcasses from 115 steers, to predict total kilograms primal lean. With the same technique, Kamuah *et al.* (1996) found correlations of 0.70, 0.82 and 0.74 between observed and estimated % lean, fat and bone in the carcass. The most complete image analysis system for beef cattle was proposed by Borggaard *et al.* (1996). In-line analysis equipment could determine conformation, fatness, fat colour, the % saleable meat and the cross section area of the rib eye. No references were found where the image analysis technique was applied on live cattle.

Density

This is probably one of the oldest method to estimate body composition (credited to Archimedes). The volume of the body is estimated by the amount of water or gas that is displaced when the subject is submerged in the medium. The weight divided by that volume and expressed in relation to the reference standard, usually water at 20°C, gives the density of the subject. Another technique is to measure the subject first in air and then submerged in water at the reference temperature. The specific gravity is the weight of the subject in air divided by the difference between the weight in air and the weight under water.

According to Miles (1982) there are few, if any, reports of successful application of this method with living cattle, although the technique proved successful to measure the composition of living humans. Some of the major problems with living animals are the tractability of the animals, the necessary corrections for lung volume and the variable content of the digestive system, especially the presence of air spaces.

Density can also be measured to estimate the composition of rib-cuts or carcasses, because these objects can easily be measured in air and in water, without the problems encountered with live animals (Torreele and Verbeke, 1966; Ledger *et al.*, 1973; Alhassan *et al.*, 1975; De Campeneere *et al.*, 1999b). Waldman *et al.* (1969) and Gil *et al.* (1970) indicated that this technique has a low predictive value when fat content is lower than 20 %.

Adipose-cell size

This method has been proposed by Robelin (1982a) to estimate body fat. The technique is most useful with slaughtered animals, but can also be applied on live animals after biopsy. A mean volume of the adipocytes is calculated, by counting and measuring the size of the cells from samples of the different adipose tissues (Robelin 1981b). This technique was applied on 12 dry cows and seemed to be as successful as deuterium dilution to estimate body fat. Due to the biopsy needed for sampling the fat tissue, this method is not useful for practical or industrial purposes.

Dilution technique

The dilution technique is based on a constant relationship between the empty body water volume and the other components of the body of the animal. If one can measure the amount of body water and the body weight, the body composition can be estimated (Reid *et al.*, 1955; Bartle and Preston, 1986). Estimating the volume of body water consists in injecting a marker that distributes quickly throughout the total body water compartment. Knowing the quantity of marker infused and comparing the concentration of the marker in the compartment before infusion and after equilibrium

has been reached, the volume of the compartment can be calculated. Hence, the total body water and from that the fat-free EBW can be estimated and consequently the lipids and the protein mass in the EB. Based on their study, Rule *et al.* (1986) concluded that before using any prediction equation for calculating body composition of cattle from *in vivo* measurement of dilution space, the equations should be tested with a sub-sample of the cattle population its use is intended for. Hammond and Waldo (1985) also decided that separate prediction equations might be required for different breeds.

It is obvious that a marker used for infusion should not be toxic and should not have any physiological effect; it should diffuse rapidly and homogeneously over the total compartment, it should not be metabolisable and preferably not foreign to the body and there should be an accurate and convenient method to determine its concentration in the sample taken from the compartment (mostly a blood sample).

According to Geerken *et al.* (1988) dilution techniques for measuring body water are recognised as most effective to predict *in vivo* body composition. The two mainly used markers are urea and labelled water, either deuterium or tritiated water, but in the past also other markers have been evaluated, such as antipyrine and N-acetyl-1,4-aminoantipyrine (Topel and Kauffman, 1988).

The dilution technique has not only been applied with different markers, but also on several species: research has been done with nursing foal (Geerken *et al.*, 1988), non lactating mature cows from different genotypes (Robelin, 1982a; Ferrell and Jenkins, 1984c), beef steers (Arnold *et al.*, 1985; Hammond *et al.*, 1988), Holstein steers (Velazco *et al.*, 1997), lambs (Bartle *et al.*, 1985), goats (Benjamin *et al.*, 1993), dogs (Painter, 1940), cats (Kornberg *et al.*, 1952) and men (Bradbury, 1961).

Labelled water dilution

Deuterium oxide is more often used as a marker than tritiated water because of the radioactivity of the latter.

Arnold *et al.* (1985) mentioned three approaches to determine body water content after injection with labelled water.

The first approach is the same as the one mostly used with urea, namely calculating the volume of the body water by dividing the amount of tracer injected by the concentration of the tracer in the blood sample when equilibrium has been reached. It is postulated that the concentration of the marker at equilibrium (Ce) is similar to Co, being the theoretical concentration of the marker in the total compartment at the time of infusion. This approach does not take into account the half-life time of labelled water in the body. The calculated dilution space is generally overestimated with 10 to 15 % (Robelin, 1984).

The second approach is based on serial measurements of the concentration of the tracer in the body water, after equilibrium has been reached, from which the concentration of the tracer at the moment of injection can be extrapolated. With this approach, also called the one-compartment model, the calculated dilution space is still overestimated with 3 to 5 % (Robelin, 1982b).

The two-compartment model or third approach only differs from the second in the statistical analysis of the dilution. The model is based on the assumption that there is a difference in diffusion between two compartments being the total body water and the

gastro-intestinal tract. Robelin (1984) and Arnold *et al*. (1985) concluded that the two-compartment model was of no greater value than the one-compartment model.

The amount of deuterium injected varies between 0.1 g/kg LW (Leme *et al.*, 1995) and 0.5 g/kg LW (Robelin *et al*., 1989), while the blood samples are collected up to 48 (Robelin *et al.*, 1989) or 72 hours (Arnold *et al.*, 1985) after infusion.

The equations of some experiments estimating the body composition with deuterium dilution are shown in Table II.3.2.

The three equations of Robelin *et al.* (1989) in Table II.3.2 for the three breeds were analysed within one model. The equations were significantly different between the breeds, but their intercept was not significantly different from zero, in contrast to other experiments. The SD mentioned in the table is the SD of the model, including the breed effect. These breed differences confirm the statements of Hammond and Waldo (1985) and Rule *et al*. (1986) mentioned above. The main factor influencing the results of infusion techniques is the variation in gastro-intestinal tract content, which contains a large amount of water, especially in ruminants. A major error is therefore introduced in the estimation of the empty body composition if total body water is assumed to be equal to empty body water. To minimise the influence of the gut content, animals should be fasted for at least 12 hours before infusion. Many mathematical models have been used to predict the water volume in the gut (*e.g.* the above-mentioned two-compartment model), but Arnold *et al.* (1985) have proven that a good prediction of gut water is still required.

Arnold *et al*. (1985) concluded that the D_2O technique is not accurate enough to estimate the composition of an animal (for scientific purposes) at a certain moment, but the technique can be considered useful to detect relative differences in body composition at different times for example, during an experiment. Robelin *et al*. (1989) considered the technique most interesting to measure the change in body fat and protein in the same animal during an experimental period. As this technique is very laborious and time-consuming it is only relevant for scientific purposes. Robelin (1982a) compared deuterium infusion with the adipose cell size technique and concluded that both techniques have comparable predictive value, but the latter is easier, cheaper, faster and independent of the gut content.

Urea dilution

Urea fits well in the conditions mentioned above to be a good marker. Moreover, urea diffuses very fast over the total body water content. According to Preston and Kock (1973) in cattle and Meissner *et al.* (1980) in bulls, after 9-10 and 10 minutes respectively, equilibrium in the total empty body water compartment is reached. Plasma urea concentrations are then nearly the same as those obtained when plasma urea disappearance curves are extrapolated back to time of injection.

Table II.3.2: Equations relating TBW (total body water; kg), EBWa (empty body water; kg), EBF (empty body fat; kg) and EBP (empty body protein; kg) with DS (deuterium space; l) and LW (live weight; kg) or EBW (empty body weight; kg)

Author	Compartment	Equation	R^2	SD
Robelin *et al.* (1989): 20 Holstein cows	TBW	= 0.979*DS	n.r.[†]	7.0
Robelin *et al.* (1989): 9 Charolais cows		= 0.951*DS	n.r.	7.0
Robelin *et al.* (1989): 10 Limousin cows		= 0.995*DS	n.r.	7.0
Robelin (1981a and c): 21 Charolais and 21 Friesian		= 0.968*DS	n.r.	4.6
Crabtree *et al.* (1974): 6 Friesian and 6 Holstein x Friesian steers		= 87.14+0.59*DS	0.85	10.5
Ferrell and Jenkins (1984c): 12 cows of four different types	EBWa	= 27.4+0.773*DS[‡]	0.92	11.0
Robelin *et al.* (1989): 20 Holstein cows	EBF	= 0.905*LW-1.140*DS	n.r.	7.2
Robelin *et al.* (1989): 9 Charolais cows		= 0.798*LW-0.975*DS	n.r.	7.2
Robelin *et al.* (1989): 10 Limousin cows		= 0.815*LW-1.047*DS	n.r.	7.2
Robelin (1981a and c)		= 0.769*LW-0.943*DS	n.r.	5.4
Crabtree *et al.* (1974)		= -99.7+0.86*LW-0.68*DS	0.87	13.0
Ferrell and Jenkins (1984c)		= -25.2+0.952*EBW-1.045*DS[‡]	0.86	15.7
Robelin *et al.* (1989): 20 Holstein cows	EBP	= 0.0902*LW+0.0727*DS	n.r.	2.7
Robelin *et al.* (1989): 9 Charolais cows		= 0.1354*LW+0.0314*DS	n.r.	2.7
Robelin *et al.* (1989): 10 Limousin cows		= 0.0617*LW+0.1530*DS	n.r.	2.7
Robelin (1981a and c)		= 0.124*LW+0.058*DS	n.r.	2.6
Crabtree *et al.* (1974)		= 19.31+0.1875*DS	0.74	4.7
Leme *et al.* (1995): Nelore steers		= 1.94+0.12*LW+0.046*DS	n.r.	n.r.

[†] n.r. = not reported

[‡] the deuterium space considered to be related to empty body water space based on a two pool model

For determining the total body water volume, the same approaches can be used as the ones used for deuterium dilution. But, due to the fast dilution of the urea the first approach is most commonly used. In general, samples are taken at 12 minutes post mean infusion time because then the highest correlations are found between US (urea space) and body compositional characteristics (Kock and Preston, 1979). US is then calculated as a percentage of LW or EBW using the following formula (Bartle *et al.*, 1983):

US (%) = mg of urea infused/(change in plasma urea concentration x W x 10)

Change in plasma is expressed in mg/100 ml plasma and W (kg) can be LW, EBW or fasted live weight (fLW). When calculating the US as a volume, the W-factor is removed from the equation.

In contrast with the deuterium technique where the amount of deuterium infused per kg body weight varies widely between experiments, it seems generally accepted to infuse urea at 130 mg/kg body weight. More disagreement concerns the removal of the feed and/or the water. Preston and Kock (1973) removed the feed and water the evening before infusion of the steers. Velazco *et al.* (1997) only removed feed 24 hours before infusion of Holstein steers, while Bartle *et al.* (1987) infused non fasted steers and heifers.

According to Bartle and Preston (1986) urea only diffuses very slowly into reticulo-ruminal water and the quantity diffused in that segment within 12 minutes after infusion is of no significant importance. This could explain why *e.g.* Preston and Kock (1973), who fasted their animals, and *e.g.* Bartle *et al.* (1987) who did not remove the feed before infusion, both found satisfying correlations. Urea space is thus considered to estimate EBWa (empty body water) plus an overestimation with the volume of urine produced during the dilution time (Bartle and Preston, 1986). This is in disagreement with Meissner (1976), who found that urea space at equilibrium measured total body water and not merely water exclusive of digestive tract. The volume of diffusion, usually called urea space (US) can be used to estimate EBWa, EBP (empty body protein) and EBF (empty body fat).

The short equilibration time, the limited handling of the animals (only two samples) and the relatively simple analyses of urea are the most important advantages of this method (Bartle *et al.*, 1983).

Results of different experiments using urea infusion in estimating body composition are shown in Table II.3.3. The positive effect of including live weight in the equation to estimate body components is shown by giving the equations with and without LW. From Table II.3.3 it can be concluded that the estimation of the water and protein content is quite reliable, while the precision to estimate the fat component from urea space is less satisfying.

Reid and Robb (1971), Hammond *et al.* (1988), Velazco *et al.* (1997) and others found no added benefit in using the urea dilution technique over LW or EBW to estimate carcass composition. Both found very high R^2-values between EBW and carcass or body water (0.97 and 0.99 respectively) and protein (0.96 and 0.99 respectively), while the R^2 with fat were somewhat less good (0.84 and 0.74 respectively). Urea dilution is less time-consuming than deuterium, but for industrial

use, this technique is still too laborious. Wells and Preston (1998) investigated the possibility for practical use of the urea dilution technique in a scientific project. They found no adverse effects of repeated urea dilutions on performances, and the technique accurately evaluates cattle of different breeds. This technique is useful for scientific purposes. It requires no expensive equipment and measurement does not take too much time.

Urinary creatinine excretion (UCE)

Some fifty years ago different authors found evidence that urinary creatinine excretion was highly correlated to body weight or to the lean tissue of the animal. The daily excretion of creatinine is not affected by protein intake. The differences between individual animals are larger than the differences between the excretions from day to day in the same animal. As the lean tissue of an animal can be considered not to change markedly from day to day, it can be expected that creatinine excretion may be of value in predicting differences in the lean tissue content of animals (Lofgreen and Garrett, 1954). Faichney *et al.* (1995) however, found that creatinine excretion could be influenced by diet. Dapoza *et al.* (1999) concluded that dietary protein level only scarcely influences the creatinine excretion if total urine production is collected. Borsook and Dubnoff (1947) found that 98 % of the creatine reserves of the animal are present in the muscles, mainly in the form of phosphocreatine. From that creatine approximately 2 % is daily converted into creatinine, which is excreted in the urine.

Schroeder *et al.* (1990) studied the relationship between UCE and lean body mass (LBM), EBP and skeletal muscle protein (SMP) in beef steers. The R^2-values of the equations predicting the LBM, EBP and SMP from urinary creatinine excretion (UCE) were 0.84, 0.81 and 0.75 respectively. When fLW was added as an independent variable, the accuracy of the predictions greatly improved towards 0.98, 0.98 and 0.95. Lofgreen and Garret (1954) found a correlation of r = 0.67, between the creatinine excretion and the percentage separable lean in the soft tissue of the 9-10-11th rib-cut in a study with 18 Hereford steers. The lean in the rib-cut was on its turn highly correlated (r = 0.90) with total lean in the carcass.

Van Niekerk *et al.* (1963) stated after research with 65 sheep that total EBP, EBW and the fat-free mass of the ingesta-free body were each highly correlated (each time r = 0.97) with the amount of creatinine excreted in the urine. Forbes and Bruining (1976) found a higher correlation, but also a higher standard deviation (r = 0.99, SD = 2.57 kg) between LBM and UCE with 34 adults and children, although they estimated the LBM by the ^{40}K technique (see later).

For this technique, a total collection of the urine over at least a 24-hour period and a simple analysis method is needed. For animals the urine is usually collected over several days and a composite sample is analysed. Except for the collection of the urine this technique is very simple, little time-consuming, requires no high investments and gives most satisfying correlations.

Table II.3.3: Equations relating EBWa (empty body water; kg), EBP (empty body protein; kg), EBF (empty body fat; kg) and HCF (half carcass fat; kg) with US (urea space; l) and LW (live weight; kg) and the R^2-values

Author			R^2			R^2
Ru	EBWa	= 20.7+0.94*US	0.83	EBWa	= 31.3+0.16*US+0.34*LW	0.98
Ha		= 33.8+0.90*US	0.92		= 35.4+0.54*US+0.16*LW	0.95
Hb		= 5.5+0.97*US	0.89		= 17.1+0.57*US+0.15*LW	0.93
Hc		= 20.6+0.93*US	0.95		= 6.7+0.37*US+0.33*LW	0.97
B		= 22.0+0.93*US	0.95			
M		= 1.02*US	n.r.[†]			
Ha	EBP	= -4.3+0.31*US	0.90	EBP	= 5.0+0.14*US+0.072*LW	0.94
Hb		= -1.6+0.32*US	0.85		= 3.8+0.13*US+0.072*LW	0.92
Hc		= -1.2+0.31*US	0.95		= -5.7+0.13*US+0.10*LW	0.97
Ru	EBF	= -64.1+0.87*US	0.66	EBF	= -48.3-0.29*US+0.50*LW	0.95
Ha		= -56+0.69*US	0.55		= -51.3-0.43*US+0.50*LW	0.81
Hb		= -81.6+0.97*US	0.45		= -22.7-1.07*US+0.78*LW	0.91
Hc		= -19.8+0.30*US	0.83		= -22.1+0.21*US+0.054*LW	0.84
B		= 46.4+0.14*US	0.04			
M		= 0.26*US	n.r.			
J	HCF	= 10.5+0.09*US	0.19	HCF	= -27.4+0.11*US+0.16*LW	0.39

Ru: Rule *et al*. (1986): 28 crossbred steers of 6, 12 or 18 months / Ha: Hammond *et al*. (1984 and 1988): 68 mixed-breed from 210 to 517 kg / Hb: Hammond *et al.* (1984 and 1988): 50 Angus steers from 219 to 517 kg / Hc: Hammond and Waldo (1985), Hammond *et al.* (1990): 38 Holstein steers from 143 to 404 kg / B: Bartle *et al.* (1987): 27 Hereford x Angus and 27 Chianina (27 male, 27 female) from 234 to 288 kg / M: Meissner *et al.* (1980): 20 bulls 101-772 kg / J: Jones *et al*. (1982): 25 Holstein cows and 30 steers

[†]n.r. = not reported

Hormone measurement

Some hormones play an important role in growth and development and in the metabolic rates, and may have also a major influence on body composition. Insulin, for example, regulates the carbohydrate metabolism and influences protein synthesis. In many mammalian species obesity seems to be associated with a high insulin secreting ability whereas low insulin secretion is associated with leanness. Gregory *et al*. (1980) found for cattle, as was previously proved for non-ruminants, that only age-related differences in fatness can be estimated from the insulin secretion, whereas the differences in fatness between animals of the same age are not associated with insulin secreting ability.

Other important hormones playing a role in the metabolism of growth are adrenaline and noradrenaline, growth hormone, and thyroxine and triiodothyronine.

Miles (1982) concluded that more research is needed to provide a fundamental understanding of the function of the hormones in the body and their relation to body composition. Initial simple attempts to relate plasma hormone levels to the body composition of cattle have been unsuccessful.

Whole body ^{40}K counting

Because a direct relation exists between potassium and lean body mass, and an indirect relation with body fat, body composition can be predicted by counting whole body ^{40}K. Of all naturally occurring potassium, approximately 0.012 % is made up of the radioactive isotope ^{40}K, the rest comprises the stable isotopes ^{39}K (93.2 %) and ^{41}K (6.8 %). The gamma radiation (at 1.46 MeV) emitted by the ^{40}K isotope can be measured and used to estimate total potassium content and hence total body protein and lean mass. Lohman and Norton (1968) reported the standard error for estimation of total body potassium to be 3.4 % and 4.2 % when estimating the carcass lean mass. Frahm *et al.* (1971) predicted the FFL (fat free lean mass) of beef bulls with the following equation: FFL = 36.4 + 0.0064 KC (r = 0.86), where KC is the average of two ^{40}K counts. Domermuth *et al.* (1976) found correlations between the lean cut weight and the ^{40}K counts of r = 0.72 and r = 0.69 in two experiments with swine.

The method is only accurate when precautions are taken to avoid background radiation during the measurement. Uncertainties can arise from differences in body shape and position of the subject, and from radioactivity of the gastro-intestinal contents (Frahm *et al.*, 1971). The huge and expensive apparatus needed for the counting of the potassium mainly restricts the use of this technique.

Ultrasound measurement

As the meat industry moves towards a value-based marketing, ultrasound seems to be one of the most promising techniques to estimate pre-slaughter body composition (Fursey *et al*., 1991). This technique is fast, non-destructive and relatively inexpensive (Faulkner *et al.*, 1990). Cross and Belk (1994) mention several distinct advantages of the ultrasound technique: 1) it may be used on live animals; 2) it may be used on slaughter floors before hide removal; 3) with development it may accurately predict traits related to palatability (*e.g.* marbling); 4) it offers no health hazards; 5) it

would allow complete automation of grading and remove the element of human error; and 6) with development it offers great compatibility with integrated artificial neural networking technology.

Present ultrasound techniques mostly predict the content of intramuscular fat, marbling score or *m. longissimus* area from scans on carcasses, meat or on the live animal. From these estimates regression equations can be drawn to estimate water, protein and/or fat content of the carcass or body.

The basic principle of ultrasonic estimation of body composition is the partial reflection of high-frequency sound signals passing through tissues, when encountering an interface between two tissues. The high-frequency sound signals originate from electrical pulses that are converted by a transmitter. The sound signals are passed through the tissues until reflected at an interface. A receiver then picks up the reflected signals that afterwards can be amplified and monitored on an oscilloscope. While passing through the tissues, energy is removed from the soundbeam due to different losses such as absorption, dispersion and scattering (Whittaker *et al.*, 1992). This reduction of amplitude is called ultrasonic attenuation. These losses are frequency dependent and therefore a sound of lower frequency will penetrate further into soft tissue than a higher frequency sound. For this reason a frequency of 3 Mhz is more appropriate for deeper locations in the body (area of *m. longissimus*), whereas a 5 MHz frequency is better for analysing tissues close to the body surface (fat thickness) (Houghton and Turlington, 1992).

For practical use in body composition, distinction should be made between three types of ultrasonic machines, which differ from each other in the way the results of the scans are displayed (the output mode).

The simplest form is the 'A'-mode or amplitude-mode ultrasonic technique. This machine gives a one-dimensional representation of the reflected signal by plotting the magnitude (amplitude) of the reflection against time. The result on the screen is a curve, or peaks superimposed on a time baseline, with the distance between the peaks being a measure of the thickness of the tissues. The Krautkramer is an example of an A-mode ultrasound instrument.

The 'B'-mode or brightness ultrasonic machine shows the signals on a cathode ray tube as a gray-level image. It is a two-dimensional display of dots. The different levels of gray are represented by a series of bright spots with differing distances. The distances between the spots are representative for the thickness of the tissues. The image represents a plane away from the transducer. These machines are usually equipped to move across the body of the animal and make different ultrasonic scans, which then can be monitored on a screen or stored on Polaroid film. The Scanogram is an example of a 'B'-mode ultrasonic machine.

The real-time ultrasonic machine produces a practically instant picture by rapid electronic switching from element to element. The basic principle is similar to the one explained above, but with this equipment movements of the tissues can be seen thanks to the continuous nature of the picture. It could be considered as a B-mode ultrasound, from which the images are updated at video rate. As an example the Danscanner can be mentioned.

M-mode or motion-mode is a combination of the three previous systems. It represents on the horizontal axis the elapsed time and on the vertical axis the distance from the transducer. The amplitudes of the reflections are plotted as gray-level intensities at their respective locations on the vertical axis. This technique is mostly used to display moving organs such as heart valves. No reference for estimating body or carcass composition was found using this equipment.

To interpret the pictures of the B- and real-time-mode scanners, a planimeter, linked to a computer or a microprocessor, is used to estimate depths and areas of the pictures.

The relationship between ultrasonic measurements and carcass characteristics is usually described by simple correlations but it should be kept in mind that this parameter is influenced by total variation in weight and carcass composition among the animals measured (Andersen, 1975). A literature review of correlations between ultrasonic measurement and carcass characteristics is shown in Table II.3.4.

A-mode is mainly used on meat to estimate fat and/or moisture content. One statistical result of the A-mode scan is the ultrasonic longitudinal speed. The longitudinal speed for pure fat is 1.45 m/s whereas the velocity through lean muscle is approximately 1.58 m/s (Goss *et al.*, 1979). Through bone, sound travels at approximately 3.10 m/s (Houghton and Turlington, 1992). Whittaker *et al.* (1992) found accordingly a gradual decrease of the longitudinal speed with increasing fat concentration in the meat sample ($r = -0.71$). The correlation of longitudinal speed and visual marbling was merely -0.49. Park *et al.* (1994) found respectively $r = -0.82$ and $r = -0.72$ for the same correlations. Another statistical output of the A-mode is the attenuation. Whittaker *et al.* (1992) found that, even though the correlation between ultrasonic attenuation and fat concentration was not high, the ultrasonic attenuation increased gradually as the fat concentration increased and the attenuation was more sensitive at higher frequencies.

As the statistical result of a B-mode scan, the Fourier transform (Fourier mean, standard deviation and count), fractal dimension, slope, being the attenuation of the image intensity of each column, and some parameters of intensity (mean of image intensity, standard deviation of image intensity and intensity count) can be interpreted. Whittaker *et al*. (1992) found most of the individual parameters of B-mode ultrasonic images from live animals to be better correlated with marbling score than parameters from scans on slaughtered animals. The respective R^2-values for non-enhanced live and post-mortal scans were 0.66 and 0.45, while for enhanced (output images corrected for noise) scans they were 0.46 and 0.23.

Comparing an A-mode (Sonatest) with a B-mode (Scanogram) ultrasound machine, Kempster *et al.* (1981) found more precise predictions of carcass lean and subcutaneous fat with the Scanogram than with the Sonatest. With that better B-mode ultrasound it was possible to reduce the residual standard deviation of predicted percentage lean to less than 2.0 %. Tong *et al.* (1981) also concluded the B-mode (Scanogram) to be better than the A-mode (Krautkramer). Gillis *et al.* (1973) also compared an A-mode (Krautkramer) with a B-mode (Scanogram) device in an attempt to estimate fat thickness and rib eye area in cattle. They concluded that the A-mode ultrasound technique, although the slowest one, is recommended, provided that it is

used by an experienced and competent operator. He found little difference in estimating the fat thickness, but when predicting the loin eye area the A-mode technique gave better results (Table II.3.4). This contradiction could be explained by an experiment performed by Herring *et al.* (1994) who found an important influence of the operator on the results. According to them it is necessary that all ultrasound operators undergo rigorous training and testing. Waldner *et al.* (1992) found two groups of conclusions. Some researchers found ultrasound measurements of physical dimensions and subsequent prediction of carcass characteristics to be quite accurate, while others found low correlations between carcass and ultrasound measurement for cattle and sheep and high correlations for pigs. Besides large operator effects, differences could be caused by contributions of instruments, hide thickness, haircoat length, weight and fat thickness.

With Real-time ultrasound equipment Waldner *et al.* (1992) found, in agreement with Parret *et al.* (1987) in cattle and others in swine (Kreider *et al.*, 1986) and sheep (Hopkins, 1990), that as fat thickness increased, the accuracy of the measurement decreased. Moreover, they found a tendency to overestimate thin cattle and underestimate fat cattle.

Faulkner *et al.* (1990) found real-time ultrasound to be an accurate and precise method for measuring fat thickness in live cattle. In combination with live weight and hip height, real time ultrasound can be used to predict accurately and precisely percentage of fat and bone and kilograms of fat and fat-free lean for mature cows. Perkins *et al.* (1992) and Smith *et al.* (1992) both found real-time to be a good estimator for carcass fat thickness, but the technique to estimate the *m. longissimus* area warrants further investigation.

The main disadvantage of ultrasound measurement is that the sound signals do not get deep enough into the body to have a whole cross-section scan of the body (Szabo *et al.*, 1999). For that reason techniques such as nuclear magnetic resonance and computerised tomography (see further) have higher potential for prediction of body composition. Nevertheless the technique may succeed in ranking the animals with regard to fatness in near slaughter weight range. This explains the successful application of ultrasound in breeding programmes (Szabo *et al.*, 1999). Rösler *et al.* (1996) concluded that the information from *in vivo* ultrasonic measurements for the estimation of carcass composition is of limited use and does not justify the expenditure and effort.

In recent years research on ultrasound seems to have focussed more on post mortem prediction of ribeye area and backfat thickness (*e.g.* Moser *et al.*, 1998; Griffin *et al.*, 1999).

*Table II.3.4: Review of correlations between in vivo ultrasonic scans and carcass characteristics LMA (*m. longissimus *area) and FT (fat thickness) in cattle*

	N[†]	mode	Equipment	Animals	Weight (kg)[§]	LMA	FT
Gillis *et al.* (1973)	31	A	Krautkramer	heifers	375	0.88	0.61
Hedrick *et al.* (1962)	47	A	Branson Sonoray 5	heifers + steers	308-420	0.58	0.53
Hedrick *et al.* (1962)	28	A	Branson Sonoray 5	steers	444-594	0.89	0.63
Hedrick *et al.* (1962)	57	A	Branson Sonoray 5	steers	304-442	0.78	0.43
McReynolds and Arthaud (1970)	39	A	Branson Sonoray 52	bulls	232-418	n.r.‡	0.61
McReynolds and Arthaud (1970)	24	A	Branson Sonoray 52	steers	229-387	n.r.	0.38
McReynolds and Arthaud (1970)	10	A	Branson Sonoray 52	bulls + steers	n.r.	0.95	n.r.
Tong *et al.* (1981)	356	A	Krautkramer	mixed	483 (29)	n.r.	0.54
Tong *et al.* (1981)	98	A	Krautkramer	steers	442 (37)	n.r.	0.57
Davis and Long (1962)	60	B	Branson Sonoray	steers	n.r.	0.87	0.90
Gillis *et al.* (1973)	65	B	Scanogram	heifers	375	0.56	0.65
Tong *et al.* (1981)	98	B	Scanogram	steers	442 (37)	n.r.	0.61
Watkins *et al.* (1967)	40	B	Branson Sonoray 510	mixed	450	0.57	0.80
Watkins *et al.* (1967)	40	B	Branson Sonoray 510	mixed	450	0.69	0.93
Watkins *et al.* (1967)	40	B	Branson Sonoray 510	mixed	437	0.37	0.72
Faulkner *et al.* (1990)	47	RT	Technicare	cows	446 (61)	n.r.	0.89
Miller *et al.* (1986)	50	RT	n.r.	mixed	n.r.	0.96	0.88
Parret *et al.* (1987) trial 1	27	RT	n.r.	heifers + steers	263-360	n.r.	0.81
Parret *et al.* (1987) trial 2	99	RT	n.r.	heifers + steers	n.r.	n.r.	0.43
Parret *et al.* (1987) trial 3	245	RT	n.r.	steers	260-440	n.r.	0.73
Perkins *et al.* (1992)	646	RT	Aloka	heifers + steers	490 (42)	0.60	0.75
Perry *et al.* (1989)	186	RT	n.r.	n.r.	n.r.	0.76	0.86
Perry *et al.* (1990)	53	RT	General Electric Datason	steers	n.r.	0.96	0.90
Smith *et al.* (1992)	315	RT	Aloka	yearling steers	502 (36)	0.89	0.81
Smith *et al.* (1992)	137	RT	Aloka	yearling steers	529 (37)	0.63	0.82
Stouffer *et al.* (1985)	51	RT	n.r.	heifers + steers	355-686	0.87	0.78
Waldner *et al.* (1992)	60	RT	Technicare	bulls	148-828	0.73	0.86

[†] N = number of animals involved in the trial / [‡] n.r. = not reported / [§] weight: x = mean, x (y) =mean (SD), x - y = range

Computerised tomography (CT)

This is one of the most promising techniques for the estimation of *in vivo* body composition. The apparatus is equipped with detector-cells to measure the amount of x-ray passing through the body. By rotating a x-ray source and detector 360° around the object the density of the different body tissues, at different distances of the x-ray can be calculated, based on the absorption data (Forrest and Judge, 1994). As such the density of air is -500, lung tissue has values between -200 and -100, fat tissue between -100 and 0, muscle tissue between +30 and +100 and bone between +400 and +500 (Skjervold and Vangen, 1981). A computer then calculates the density in each point and reconstructs a slice through the object. As such the composition of the slice and of the total body can be estimated.

From preliminary studies with 23 pigs, Skjervold (1982) found R^2-values between the dissection of the slice and the estimation of the composition of the slice of 0.89 for fat, 0.80 for protein and 0.85 for water. For the dissection of the total body and the estimation of total body composition by CT he found R^2-values: 0.89 for fat, 0.83 for protein and 0.82 for water. Surprisingly, the figures for the total body composition estimation were as good as these for the estimation of the slice. Skjervold (1982) concluded that with use of more than one slice and by removing the CT-values that describe the intestines, stomach etc. from the tomographic slice, the estimation can still improve. Standal (1984) believed that it would be possible to estimate carcass composition of animals with about the same accuracy as with slaughtering and dissection procedures.

One of the major advantages of CT and MRI (see further) techniques is that instead of using prediction equations, which are mostly breed and species dependent and need to be revalidated regularly, the direct measurement of tissue volume is possible independently of the shape of the body (Szabo *et al*., 1999).

In human medicine this technique is frequently used. However, as these techniques are adopted from human medicine, the size of the animals is limited to a size comparable with that of human beings. For practical use in cattle, some adaptations have to be made (Skjervold and Vangen, 1981). No references were found applying the CT technique on cattle so far. The cost, the time to make an estimate and the necessity to anaesthetise the animal before scanning are the most important factors to deal with, before this technique can become applicable in practice.

Dual-energy X-ray absorptiometry (DXA)

In recent years this new technique has evolved from an other technique, dual-energy photon absorptiometry (DPA), which was originally developed for measuring the density and the mineral mass of the bone in humans (Mitchell *et al*., 1996). The theory and methodology for measuring body composition using DXA is based on the difference in attenuation of low (38-keV) and high-energy (70-keV) x-rays by fat and other soft tissues. DXA has been tested *in vivo* on rats (Jebb *et al.*, 1996) and pigs (Mitchell *et al.*, 1996 and 1998). The technique seemed promising but only after calibration and adaptation of the software used for the experiment. For cattle, no reference was found except on analysis of beef rib sections (Mitchell *et al*., 1997). The authors concluded that further calibration is needed, but DXA appears to be a valid

approach for measuring the composition of cuts of beef where the composition is desired without deboning, dissecting or homogenising.

Nuclear magnetic resonance imaging (magnetic resonance imaging: MRI)

The elements of odd atomic number have a magnetic field, which strength and orientation can be described by vectors. Without a strong external magnetic field, these vectors are oriented randomly in space, so their summation is zero. But in a strong external magnetic field they are aligned mainly in the direction of the magnetic field's longitudinal axis and resonate at a particular frequency. After altering the direction of the atoms their magnetic field by applying a radio frequency on the transverse plane of the longitudinal axis, the magnetic vector of the atoms recover their original position, generating a small radio frequency signal which can be detected.

Practically, MRI imaging machines consist of a magnet with a central opening large enough to accommodate the subject (animal or human). That magnet applies a strong and uniform magnetic field to the subject. Because the nuclei of atoms with an odd number of protons and/or neutrons are slightly magnetic, that field aligns these nuclei. The most abundant of these nuclei is that of the hydrogen atom, consisting of one single proton, predominantly occurring in water and in the $-CH_2$ groups of free lipids such as in adipose tissue (Ettinger *et al.*, 1983). When superimposing slightly changing magnetic fields on the main field (by passing electric currents through subsidiary coils enclosing the animal), the concentration, distribution and properties of the protons in the body can be explored by measuring the electromagnetic signals radiated by the protons (Wells, 1984). MRI measurements visualise the tissues according to their observable proton density and the relaxation time. Baulain (1997) discussed the use of MRI in animal science very extensively.

As discussed before, the CT scan technique seems to be very promising for the future, but theoretically, MRI gives even more possibilities. The results of the CT scan are pixels with different Hu-values (Hounsfield units), which are than converted to different grey values. These values are solely in function of the X-ray attenuation ability of the tissue (Szabo *et al.*, 1999). In the case of MRI, the grey value of the pixels depends on several factors: proton density, velocity of the flowing body fluids and T1 and T2 (relaxation constants), which are two parameters describing some characteristics of the atoms (Szabo *et al.*, 1999).

The most important advantages of this technique according to Miles (1982) are:

- the technique is non-invasive and uses non-ionising radiation (and is as such considered as save)
- the electromagnetic radiation penetrates bone-tissue and deeply into the body without significant attenuation
- it measures the density distribution of hydrogen, the most abundant element in the body, and does so with useful tissue discrimination.

Fuller *et al.* (1984) and other authors found good images of the muscle, fat and bone portions of the sites scanned with pigs. Several authors also found good correlations between MRI measurement and body composition with pigs (see Szabo *et al.*, 1999). No data were found on predicting body composition in live cattle with MRI.

This estimation method has great potential, but unfortunately, the technique is very expensive and complex.

A special application of the MRI technique is the topical MRI, by which a particular region of the living mammalian body is focused and high-resolution spectra are obtained (Miles, 1982). The extent of the region can be made larger or smaller and its position can be manipulated to measure the concentration of important chemicals at specific positions inside the living mammalian body.

The MRI technique has first been developed for human medicine and later on, been adapted for the use on animals. MRI, also called NMR (nuclear magnetic resonance) could provide an additional *in vivo* method for assessing the meat/fat ratio and for giving information related to the chemical composition of the tissues (Worthington, 1983).

The main problem for application with cattle is the size of the adult animals. The object has to be surrounded by a magnet, which is not absolutely necessary for the CT-technique. Besides, the same problems as with CT scan have to be dealt with (sedation and cost and time of measurement). The high costs and the lack of portability exclude general and intensive application of both techniques, but they could be applied in research and breeding schemes (Szabo *et al.*, 1999). The very high precision of CT and NMR is very promising and these techniques could therefore become an alternative for total body analysis as a calibration method in experiments evaluating other estimation techniques. Before that, some serious development has to be done, especially for cattle.

Electromagnetic Scan (EMS)

Electronic meat-measuring equipment (EMME)

The concept of this technique is based on the much higher electrical conductivity of muscle than of fat. For the measurement, an electrical current passes through a coil that surrounds the animal. That current produces an alternating magnetic field, which on its turn induces eddy-currents in the animal's body. These eddy-currents generate a magnetic field that can be measured by a change of the impedance in the coil.

With pigs, Domermuth *et al.* (1976) found a $R^2 = 0.78$ between live EMME measurement in combination with shrunk body weight and the weight of carcass protein.

Although the measuring device is quite big (± 1.8 x 2.4 m), the important advantage of this technique is the speed of the measurement. While an animal passes through the central tube, which is surrounded by the coil, a small high frequency current is induced through the coil and the change in drive current characteristics is analysed. The result is a single EMME number that can be read directly from a digital dial. Thus, pigs can be measured as quickly as it is possible to move them through the instrument.

In general, the results obtained with this technique are too variable to be acceptable for use in practice (Fredeen *et al*., 1979; Mersmann *et al.*, 1984; Topel and Kauffman, 1988).

Electromagnetic Scan (EMS)

The EMME technique is now considered as 'one of the first applications of EMS' (Gwartney *et al.*, 1994). Based on the same principle as the EMME, being the difference in electrical conductivity and the dielectric properties of the different tissues, the result of an EMS-scan is a curve whereas the EMME resulted in a single figure. The initial unadjusted peak, also called the conductivity index, the smoothed peak and the area under the curve are three characteristics of the scan that can be used to predict the composition of the scanned subject (Gwartney *et al.*, 1994). The scanned subjects are mostly pig or lamb carcasses (Akridge *et al.*, 1992; Berg *et al.*, 1994a and b) or beef primal cuts. The method is fast, accurate, non-invasive and reliable (Gwartney *et al.*, 1994). Gwartney *et al.* (1992) found EMS to be an effective technology for determination of lean content in beef quarters and rounds, accounting for 85 to 90 % of the variation. Lin *et al.* (1992) found the conductivity index to be the most important variable in the prediction of lean content in larger meat cuts (chuck and round), while for the smaller meat cuts the weight was most important. No reference was found where live animals were scanned with the EMS.

Bioelectrical impedance analysis

Like many others, this method arose from initial work on humans in whom impedance measurements were used to predict total body water (Cosgrove *et al.*, 1988). The basic principle is to transmit an alternating electrical current (mostly used 800 µA at 50 kHz) from one electrode to another through the body and to measure the voltage drop that has occurred over the body, between the two electrodes, with an impedance amplifier. The conductance or resistance is dependent on the geometric configuration, the volume of the biological conductor, signal strength and frequency of the applied current. For the same amount of mass larger amounts of fat increase resistance because resistance increases as total body water decreases and with increased fat, total body water decreases (Marchello and Slanger, 1994). In practice, the electrodes used are needles inserted in the body of the animal, usually the back, or aluminium foil electrodes placed on the surface of the animal.

Generally, a four-terminal plethysmograph is used, of which electrodes are placed in live animals along the dorsal axis or on the side of the carcass at well-defined spots. Most commonly a current at 800 µA and 50 kHz is used for measurement. Hegarty *et al.* (1998) however used a multi-frequency apparatus on lamb carcasses at a current of 1.5 mA and for 378 logarithmically spaced frequencies between 1 and 916 kHz.

Marchello and Slanger (1993 and 1994) used 33 beef cows with a wide range of weight, age and fatness to evaluate the BIA technique. They found R^2-values for kg lean of 0.90 and 0.95 for live and bled animals and 0.94 and 0.92 for hot and cold carcasses, while for fat-free muscle they found 0.87, 0.93, 0.90 and 0.87 respectively. They concluded that BIA is a rapid, non-destructive method for determining the lean and fat-free muscle content of live animals and carcass beef, that it can be used as a value-based marketing tool and that BIA has potential for use in genetic selection of superior animals. Johns *et al.* (1992) found satisfying coefficients of determination (0.89 and 0.83) for predicting total lean and fat in beef carcasses using BIA. Velazco *et al.* (1999) evaluated *in vivo* BIA with Holstein steers at 3, 6, 9 and 12 month of age.

Based on the pooled data, they found correlations between resistance, reactance and impedance with carcass fat-free mass of -0.73, -0.76 and -0.73 respectively. Marchello and Slanger (1994) proved that BIA can also be used to determine weight of skeletal muscle and fat-free skeletal muscle of beef cow primal cuts. There is also potential for objective measuring of the marbling of meat.

Swantek *et al.* (1992), for live pigs and pork carcasses, Berg and Marchello (1994), for live lambs and lamb carcasses, and Slanger *et al.* (1994), for lamb retail cuts, found comparable and promising results. The measurement is rapid and simple, yet precise; it has a high repeatability, requires relatively inexpensive equipment and it doesn't require an especially skilled individual for its operation.

II.3.3 Conclusion

Several techniques exist to estimate *in vivo* body composition of animals. Depending on the experiment and the circumstances the most appropriate technique should be chosen after considering financial possibilities, the expected speed of measurement and the required accuracy of the result.

Most techniques are developed for scientific purposes, and are therefore too expensive or too time-consuming for use in practice. During the past decades several new techniques have emerged, mostly based on modern technology, such as dual x-ray absorptiometry, computerised tomography, nuclear magnetic resonance and electromagnetic scan. Although very promising for scientific and probably for industrial purposes, none of these techniques provides any help to the needs of the farmer to have an idea about the composition of his animals. He is still committed to visual assessment or linear measurement. Moreover, most of these techniques are still not adapted to be used in cattle.

The most promising technique seems the bioelectrical impedance measurement. The instrument is relatively cheap, the measurement takes very little time, is accurate, precise and has a high repeatability and above all no specialised operator is needed.

EXPERIMENTAL

PART

III Feeding trials

General outline of Chapter III

As a basic tool for determining the energy and protein standards, feeding trials are indispensable. Therefore, two feeding trials, repeated over three and two series respectively and involving a total of 333 BB dm bulls, were designed to investigate the influence of different energy and protein treatments on the performances.

The first feeding trial studied the influence of six different energy-protein combinations on the performance and on the carcass and meat quality of BB dm bulls. Therefore, two energy levels, and within each energy level, three protein levels were fed during the entire finishing period. This trial is described in **Chapter III.1:** "**Influence of dietary energy and protein levels on performance, carcass and meat quality"**.

Based on the results of that first trial, a second trial was designed. The influence of phased energy and protein feeding on the performance and carcass quality was investigated. The trial combined four different energy-protein treatments. The aim was to adjust the protein and energy feeding to the changing needs of the animals. This second feeding trial is described and its results are reported in **Chapter III.2:** "**Influence of phased energy and protein feeding on performance and carcass quality"**.

In order to make comparison between the two trials easier, the programmed and analysed energy and protein levels of the different treatments of both trials are shown together in Figure III.1.

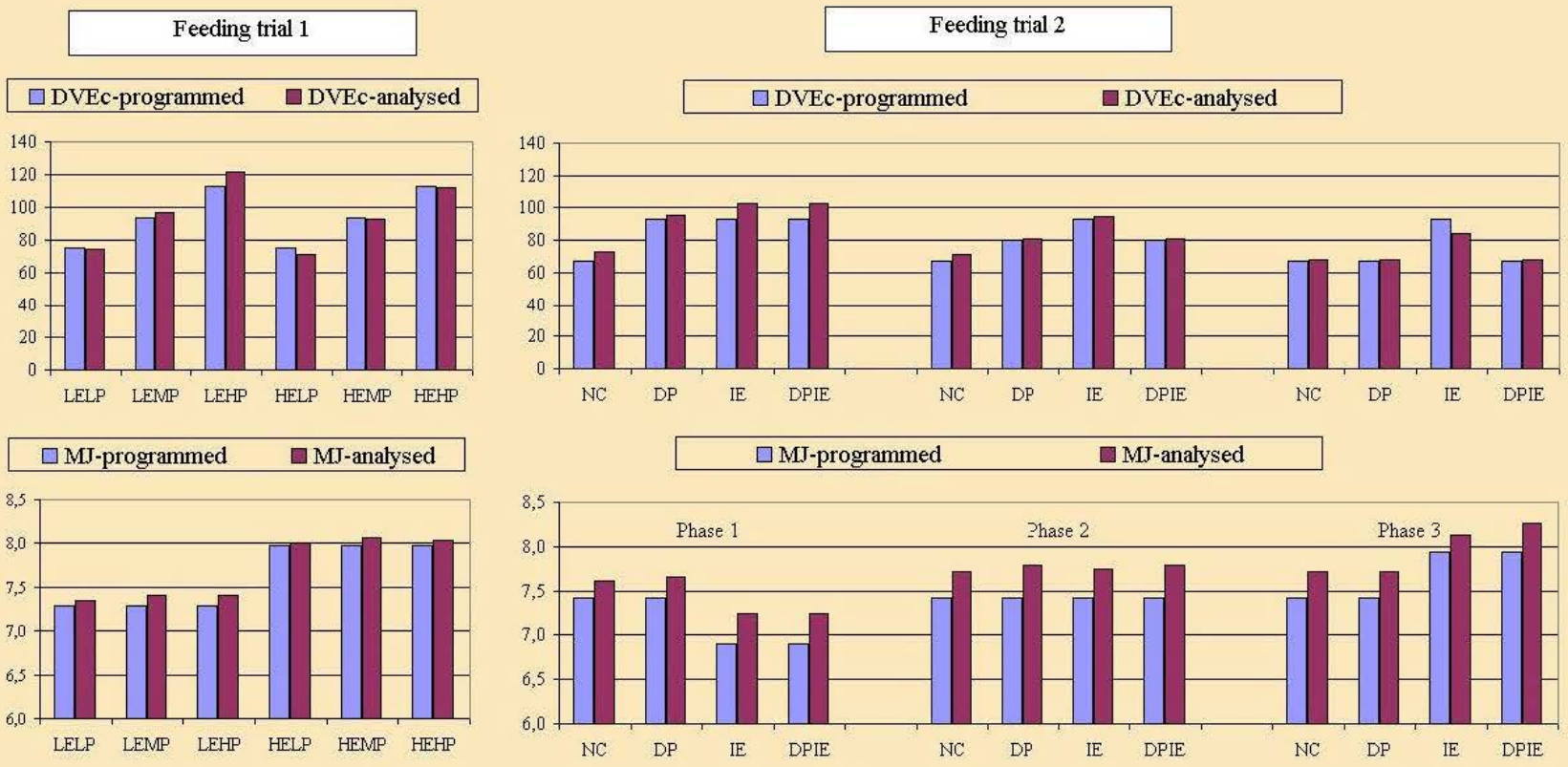

Figure III.1: Overview of the programmed and analysed DVEc (g/kg DM) and NEF (MJ/kg DM) contents of the rations of both feeding trials

LELP, HELP, LEMP, HEMP, LEHP, HEHP: low (L) or high (H) energy (E) in combination with L, moderate (M) or H protein (P)
NC, DP, IE and DPIE: negative control, decreasing protein levels, increasing energy levels and combining DP and IE

III Feeding trials

III.1 Influence of dietary energy and protein levels on performance, carcass and meat quality

Redrafted from:

"The influence of dietary energy and protein levels on performance, carcass and meat quality of Belgian White-blue double-muscled finishing bulls"

by:

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III.1 Influence of dietary energy and protein levels on performance, carcass and meat quality

III.1.1 Abstract

The effect of three protein (77, 97 and 117 g DVE (true protein digested in the small intestine) per kg dry matter (DM)) and two energy levels (7.38 and 8.03 MJ NEF (net energy for fattening) per kg DM) on the performance of Belgian Blue double-muscled finishing bulls and on the quality of their carcasses and meat was investigated. The diet, fed *ad libitum*, consisted of 65 % concentrates and 35 % maize silage on DM basis.

No significant influence was found of the energy level on the growth rate. The low protein level reduced daily live weight gain between 370 and 501 kg (1.43 kg on average *vs*. 1.60 and 1.66 kg daily; $P < 0.01$). During the second and third subperiod, protein level had no influence on daily gain. The protein content significantly influenced the growth rate during the entire period (370 to 692 kg) and the live weight at the end of the first and second period and at slaughter.

The high energy level significantly decreased DM, CP and DVE-intake, while the protein intake was positively influenced by the protein level. The feed efficiency, expressed as DM, CP and DVE was positively influenced by increasing the energy level, while increasing the protein level negatively influenced protein efficiency.

The bulls fed the high energy level lost less weight during the 20 hours fasting period before slaughter. Their carcasses were classified with a higher fatness score and a better conformation. These carcasses had also a higher fat content and a lower proportion of bone compared to the low energy groups. The cold carcass weight of the low protein groups (456 kg on average) was significantly smaller than that of the four other groups (470 kg on average). Although no differences between the six groups were found concerning the dressing proportion, this parameter was significantly influenced by the protein level (68.5 % for low protein vs. 69.1 for high protein). The SEUROP conformation was positively influenced by the protein and the energy level, while the fatness score was only influenced by the energy level (5.5 vs. 6.0 for the low and the high energy level, respectively). The differences in composition and fatness score are significant, mainly due to the small variation between animals, but their practical meaning is less important. The different protein and energy levels had also very little influence on the meat quality.

III.1.2 Introduction

In 1994, the Belgian blue (BB) breed provided 75 % of the red meat produced in Belgium (Bouquiaux and Hellemans, 1996). Within that breed the double-muscling phenomenon is extremely pronounced and it has become very common during the past decades. According to Hanset (1996) 80 to 85 % of the BB population is dm. These

animals have proven to combine high growth rates with an acceptable feed conversion, partly due to a lower feed intake capacity (Geay *et al*., 1982) caused by a reduction in the size of the digestive tract (Ansay and Hanset, 1979; Boccard, 1981). With 75 dm BB bulls within a 375 to 620 kg live weight range Fiems *et al.* (1995a) found a mean growth of 1.44 kg per day and a DM intake of 5.82 kg per kg gain on a diet of 50 % maize silage and 50 % concentrate. Minet *et al.* (1996) gathered results of 16 trials with 129 dm BB bulls fed on concentrate diets. They found an average growth rate of 1.47 kg per day and a DM conversion of 6.13, over a mean range of 330 to 576 kg. The BB dm bulls are also characterised by an excellent conformation, a very low fat content in the carcass (11-13 %) (Fiems *et al*., 1995b, Minet *et al.*, 1996) and a mean dressing percentage close to 70 % (Fiems *et al*., 1990; Uytterhaegen *et al.*, 1994; Fiems *et al.*, 1995b). The combination of these characteristics suggests that the dm animals could have specific nutritional requirements. As such, Boucqué *et al.* (1984) found that the crude protein (CP) content in the diets for dm bulls (range 350 to 650 kg) should exceed 140 g/kg, while a content of 120 g/kg was found to be sufficient for finishing BB nc bulls (Boucqué *et al.*, 1980b). Fiems *et al*. (1990) emphasised the need for a high energy density in the diet for dm animals. However, neither Boucqué *et al.* (1984) nor Fiems *et al.* (1990) investigated the existence of an interaction between the dietary energy and protein levels.

In experiments with Israeli Friesian bulls Holzer *et al.* (1986) investigated the effect of protein supplementation from 90 to 140 g CP per kg DM within two levels of energy (9.6 and 11.3 MJ metabolisable energy per kg DM). The protein supplementation had a positive effect on daily live weight gain during the first period of the experiment. Prior *et al.* (1977) found an increased average daily gain and feed efficiency with an increase of the CP level from 100 to 115 g per kg DM, while a further increase towards 130 g CP showed no advantages. The same authors found more effects of an increased energy level, such as increased marbling score, quality grade, fat thickness and yield grade within the small type of cattle, namely Angus-Hereford crossbred, than in the large types, Charolais and Chianina.

Berge *et al*. (1993) found an increased DM content and a decreased lipid content in the *m. longissimus dorsi* as the protein supply increased from a low to a high level. Bailey (1989) only found a similar influence of protein on lipid content, not on DM content. Furthermore a positive influence of the energy level on the intramuscular fat content could be expected.

In this trial the effect of CP contents higher than 140 g/kg was investigated. This was done at two different energy levels, not only to investigate the effect of energy and protein, but also the interaction between these factors.

III.1.3 Material and methods

Animals and management

During three consecutive years a total of 230 BB dm bulls were finished in order to investigate the influence of different protein and energy levels in the diet on performance and on carcass and meat quality. The animals were selected from the market supply based on their age (younger than 14 months), weight (first year between

300 and 350 kg, second and third year between 275 and 325 kg), health, the quality of their legs and conformation. Live weight at purchase averaged 309 kg (SD 25). After an adaptation period of about 1.5 month with a diet based on maize silage, grass silage and concentrates, the animals were divided into six homogeneous groups based on body weight (determined on three consecutive days), growth rate during the adaptation period, body conformation, age in days, the weight/age ratio and the standard deviation of the mean growth and weight of each group. During the trial, the animals were confined in straw-bedded loose houses and were weighed once every four weeks and on two consecutive days at the end of a subperiod (day 84 and 168). At the end of the trial, the animals were weighed on three consecutive days, with the third weighing after a fasting period of 20 hours. The experiments averaged 228 days.

Feed characteristics

Once daily, the bulls were fed *ad libitum* a diet consisting of 35 % maize silage and 65 % concentrate on DM basis. Water was freely available. The concentrate and maize silage intake were recorded daily for each pen (two years two pens and one year three pens for each treatment).

Table III.1.1: Ingredients of the concentrates (kg/tonne)

Energy level	LE[†]			HE		
Protein level	LP	MP	HP	LP	MP	HP
Sugar-beet pulp	200	220	240	-	236.2	271
Wheat	107	-	-	426	200	93
Pollards	114	34	12	-	-	-
Tapioca	150	150	150	144	-	-
Malt sprouts	150	132	102	51	-	-
Coconutmeal	33.6	149	76	-	250	250
Rapeseedoil meal	9	142	142.5	-	54	
Soya-bean meal	-	-	-	-	24	65.5
Protected soya-bean meal	-	56.6	162	-		76
Maize glutenfeed	117.1	-	-	238	103.3	112.6
Beef tallow	-	-	-	15	15	15
Beet molasses solubles	80	80	80	80	80	80
Trace elements	15.4	15.4	15.4	15.4	15.4	15.4
Vitamin mix (A, D_3 and E)	8.6	8.6	8.6	8.6	8.6	8.6
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Limestone	12.8	9.9	9	19	11	10.3
Feed phosphate	-	-	-	3.8	-	-

[†] L, M or H combined with E or P = low, moderate or high energy or protein level

All six groups received the same maize silage, but the concentrates differed in energy and protein content. Within two energy levels (high: HE and low: LE), three protein levels were realised (high: HP, medium: MP and low: LP). The mean NEF (net energy for fattening) content of the LE and HE diets were respectively 7.38 and 8.03 MJ per kg DM, while the three protein levels in the diets averaged 77, 97 and 117 g DVE

(true protein digested in the small intestine) per kg DM or respectively 127, 153 and 172 g CP per kg DM. The ingredients of the concentrates are shown in Table III.1.1.

Chemical composition (Weende scheme) and nutritive value of the concentrates and the maize silage are shown in Table III.1.2. As the maize silage was different for each year, silage composition and values are listed per year.

Dry matter of the feedstuffs was determined by drying in a ventilated oven at 65°C, grinding through a 1 mm sieve, followed by oven drying at 103°C during 4 hours. For maize silages, DM content was further corrected for losses of volatile substances according to Dulphy and Demarquilly (1981). Crude protein was determined according to the EU method (Anonymous, 1993) using an automatic Kjeltec 1035 auto sampler system (Foss Benelux, Amersfoort, The Netherlands). Crude fat was determined according to the EU method (Anonymous, 1984 (method A)) being a 6 hour Soxleth extraction (petroleumether). Crude Fibre was determined with a Fibertec apparatus (Foss Benelux, Amersfoort, The Netherlands) as described by the EU method (Anonymous, 1992). Ash was determined in a furnace at 550°C. Nitrogen free extract was determined by difference. DVE and OEB values were calculated based on the crude protein content, after *in sacco* measurements of protein degradability in the rumen and intestinal digestibility of the rumen undegradable protein fraction, using 4 lactating Holstein cows fitted with a cannula in the rumen and in the proximal duodenum (Tamminga *et al.*, 1994). NEF (VEVI) of the feedstuffs, expressed in MJ, was determined according to Van Es (1978), based on chemical composition and digestion coefficients *in vivo* determined with 5 wethers.

Carcass and meat quality

After a chilling period of 24 hours post slaughtering, the characteristics of the carcasses were determined. The carcasses were classified according to the SEUROP classification scheme (Anonymous, 1991a). The dressing proportion was calculated as the ratio cold carcass weight over fasted live weight. The carcass composition (bone, fat and meat) was assessed by dissection of the 8th rib-cut (Verbeke and Van de Voorde, 1978), while the *m. longissimus thoracis* (LT) surface was estimated using a digitizer and a specific computer program to calculate the surface of the muscle starting from a picture of it.

To assess the quality of the meat, a sample of the LT was taken at the 8th rib interface 24 hours *post mortem*. pH was measured on the excised meat on a depth of 1 cm, using a meat electrode. Measurements were corrected for temperature differences. Water holding capacity (WHC) was determined by placing approximately 300 mg homogenised meat on a filter paper between two cover glasses under a pressure of 1 kg during 5 minutes. The difference between the areas, as determined by a planimeter, of the pressed meat and the wet area on the filter paper is a measure for the exudative juice or WHC. The colour (CIELAB L*, a* and b* values) of the meat was measured using a Labscan II (HunterLab, Hunter Associates Laboratory, Inc., Resont, Virginia, USA; light source D65, 10° observer, 0°/45° geometry). The spectrocolorimeter measures the percentages of light reflected from 400 to 700 nm, at intervals of 10 and 20 nm. The colour was determined twice for each sample. Colour was calculated as the mean of these 2 measurements. Shear force values (N) were determined at the laboratory of the

Department of Animal Production of Ghent University. Steaks (width 2.5 cm) were aged for 8 days at 2°C, vacuum packed and frozen until analysis. Steaks were thawed overnight, heated in open plastic bags in a waterbath at 75°C for 1 hour and cooled under running tap water. Cylindrical samples (n = 10-15, diameter 1.27 cm) were taken parallel to the fibre direction and sheared perpendicular to the fibre direction with a triangular Warner Bratzler shear mounted on an Instron 1140 Food Tester (Instron Ltd., High Wycombe, UK). Meat composition (moisture, fat and protein) was estimated using NIRS (Near Infrared Reflectance Spectroscopy) (De Boever *et al.*, 1992).

Statistical analysis

The results were analysed using a 2x3 full-factorial design with energy (E), protein (P) and year (Y) as the main factors. Only the interaction ExP is listed in the tables. The significance of the differences between groups was based on the Duncan test (P = 0.05). The statistical units were individual data for live weight, live weight gain and carcass and meat quality parameters, while pen data were the statistical unit for intake and feed efficiency.

III.1.4 Results

Animal performance

Table III.1.3 shows the effect of the different protein and energy levels on live weight and growth rate. In both the HE and the LE levels, the growth rate of the LP groups was significantly lower during the first 84 days, in comparison with the MP and HP groups. The animals fed the LP level grew on average 1.43 kg while the other four groups averaged 1.63 kg per day. As a consequence of that lower growth rate, live weight after 84 and 168 days and at slaughter were significantly affected by the protein level. During the following periods no effect of the protein level was recorded on daily gain.

The energy level had no significant influence on live weight or on growth rate. No significant interaction between energy and protein was found for any of the subperiods, but for the total period the energy x protein interaction was significant. Within the HE group the HP group had a significant higher final weight than the MP group, which was in turn significantly different from that of the LP group. Differences were less pronounced in the LE group where the LP group was only significantly different from the MP group and surprisingly not from the HP group. The higher growth rate of the HEHP (1.49 kg per day) group in comparison with that of both LP groups (1.37 kg per day) was the only significant difference concerning growth rate for the total finishing period.

Energy was however an important factor affecting feed intake and feed conversion. Table III.1.4 shows a significant effect of a higher energy level on DM, CP and DVE intake for the three subperiods and for the total finishing period. For the total period, the mean daily DM intake of the HE groups was 540 g or proportionately 6 % lower than that of the LE groups. Comparable results were found for the daily intake of CP and DVE, namely 160 (12 %) and 124 g (15 %) respectively. The energy level only influenced the energy intake during the first period, while for the total period no significant effect was found.

Table III.1.2: Chemical composition and nutritive value of the feeds and the rations

	Concentrates						Maize silage		
Energy level	LE			HE					
Protein level	LP	MP	HP	LP	MP	HP	Year 1	Year 2	Year 3
Chemical composition									
Dry matter (g/kg)	869	871	869	865	876	877	380	375	307
Composition of DM (g/kg DM)									
Crude protein	157	204	233	152	186	214	74	74	81
Ether extract	22	31	24	37	58	57	27	24	31
Crude fibre	106	120	113	52	109	116	178	204	215
NFE	631	555	540	684	568	529	672	645	638
Ash	84	90	90	74	81	85	50	53	45
Nutritive value									
DVE[H] (g/kg DM)	91	124	157	83	112	142	57	57	58
OEB[I] (g/kg DM)	10	23	19	15	21	23	-43	-38	-34
NEF[§] (MJ/kg DM)	7.76	7.87	7.86	8.79	8.86	8.82	6.92	6.43	6.30

	Rations					
Energy level	LE			HE		
Protein level	LP	MP	HP	LP	MP	HP
Crude protein (g/kg DM)	129	159	178	126	148	166
DVE (g/kg DM)	79	101	122	74	93	112
OEB (g/kg DM)	-7	1	-1	-4	0	1
NEF (MJ/kg DM)	7.35	7.41	7.41	8.01	8.06	8.03

Data are mean values of nine analyses for concentrates (three pooled samples per year, three experimental years) and three analyses for maize silage (three pooled samples per year)

[H] DVE = true protein digested in the small intestine

[I] OEB = degraded protein balance

[§] NEF = net energy for fattening

Table III.1.3: Influence of energy (E) and protein (P) level and year (Y) on live weight and growth rate

Energy level	LE			HE			Pooled	P-value			
Protein level	LP	MP	HP	LP	MP	HP	SD	E	P	ExP	Y
Number of bulls	39	38	37	38	39	39					
Experimental days	229	228	228	228	228	228					
***Live weight** (kg)*											
Initial	371	370	370	370	371	370	30	0.94	0.99	0.96	**0.00**
Day 84	494^{ab}	508^{bc}	507^{bc}	486^{a}	502^{abc}	512^{c}	37	0.51	**0.00**	0.48	**0.00**
Day 168	616^{ab}	633^{bc}	629^{abc}	611^{a}	624^{abc}	637^{c}	40	0.68	**0.01**	0.42	**0.00**
Final	684^{ab}	701^{cd}	691^{abc}	679^{a}	693^{bc}	706^{d}	27	0.88	**0.00**	**0.00**	**0.00**
***Growth rate** (kg/day)*											
Day 0-84	1.46^{a}	1.64^{bc}	1.63^{bc}	1.39^{a}	1.56^{b}	1.69^{c}	0.25	0.25	**0.00**	0.09	**0.00**
Day 85-168	1.45	1.48	1.45	1.49	1.45	1.48	0.23	0.71	0.98	0.50	**0.00**
Day 169 to end	1.14	1.26	1.16	1.16	1.18	1.23	0.38	0.97	0.62	0.47	**0.00**
Total period	1.37^{a}	1.47^{ab}	1.43^{ab}	1.37^{a}	1.42^{ab}	1.49^{b}	0.21	0.98	**0.03**	0.26	**0.00**

^{a,b,c,d}: means in a row with different superscripts are significantly different ($P < 0.05$)

Table III.1.4: Influence of energy (E) and protein (P) level and year (Y) on daily intake

Energy	LE			HE			Pooled	P-value			
Protein	LP	MP	HP	LP	MP	HP	SD	E	P	ExP	Y
***DM** (kg)*											
Day 0 - 84	8.14^{ab}	8.42^{b}	8.32^{b}	7.76^{a}	7.90^{ab}	8.12^{ab}	0.46	**0.02**	0.29	0.65	0.44
Day 85 - 168	9.77^{ab}	9.96^{b}	9.60^{ab}	9.08^{a}	9.12^{a}	9.23^{a}	0.64	**0.00**	0.91	0.54	**0.04**
Day 169 - end	9.80^{ab}	10.01^{b}	9.50^{ab}	9.17^{a}	9.43^{ab}	9.40^{ab}	0.67	**0.04**	0.48	0.64	0.25
Total period	9.15^{ab}	9.47^{b}	9.19^{ab}	8.61^{c}	8.73^{ac}	8.84^{ac}	0.50	**0.00**	0.48	0.43	0.10
***CP** (kg)*											
Day 0 - 84	1.04^{a}	1.33^{b}	1.46^{c}	0.98^{a}	1.15^{d}	1.33^{b}	0.19	**0.00**	**0.00**	0.18	0.08
Day 85 - 168	1.25^{a}	1.58^{b}	1.70^{c}	1.14^{d}	1.35^{e}	1.51^{b}	0.21	**0.00**	**0.00**	0.13	0.15
Day 169 - end	1.24^{a}	1.58^{b}	1.69^{c}	1.13^{d}	1.38^{e}	1.56^{b}	0.22	**0.00**	**0.00**	0.39	0.25
Total period	1.17^{a}	1.50^{b}	1.63^{c}	1.08^{d}	1.28^{e}	1.46^{b}	0.20	**0.00**	**0.00**	**0.03**	0.07
***DVE**† (kg)*											
Day 0 - 84	0.63^{a}	0.83^{b}	1.00^{c}	0.57^{d}	0.72^{e}	0.87^{b}	0.16	**0.00**	**0.00**	0.32	0.64
Day 85 - 168	0.76^{a}	0.99^{b}	1.16^{c}	0.66^{d}	0.83^{e}	1.00^{b}	0.18	**0.00**	**0.00**	0.21	0.33
Day 169 - end	0.76^{a}	0.99^{b}	1.15^{c}	0.67^{d}	0.85^{e}	1.03^{b}	0.18	**0.00**	**0.00**	0.54	0.44
Total period	0.71^{a}	0.94^{b}	1.11^{c}	0.63^{d}	0.79^{e}	0.97^{b}	0.17	**0.00**	**0.00**	0.06	0.33
***NEF**† (MJ)*											
Day 0 - 84	59.52^{a}	62.15^{ab}	61.39^{ab}	61.66^{ab}	64.08^{ab}	65.67^{b}	4.14	**0.04**	0.14	0.67	**0.00**
Day 85 - 168	71.61	73.47	71.06	72.57	73.47	74.37	5.18	0.52	0.89	0.58	**0.00**
Day 169 - end	71.68	73.75	70.02	72.99	75.82	75.20	5.87	0.14	0.43	0.78	**0.01**
Total period	66.98	69.81	67.88	67.33	70.16	71.19	4.35	0.48	0.24	0.58	**0.00**

^{a,b,c,d,e}: means in a row with different superscripts are significantly different ($P < 0.05$)

† see footnote Table III.1.2

Table III.1.5: Influence of energy (E) and protein (P) level and year (Y) on feed conversion

Energy level	LE			HE			Pooled	P-value			
Protein level	LP	MP	HP	LP	MP	HP	SD	E	P	ExP	Y
DM *(kg/kg growth)*											
Day 0 - 84	5.66[a]	5.23[abc]	5.18[bc]	5.59[ab]	5.08[c]	4.87[c]	0.47	0.30	**0.00**	0.50	**0.04**
Day 85 - 168	6.71	6.75	6.84	6.29	6.28	6.25	0.67	**0.02**	0.87	0.93	0.08
Day 169 - end	8.67	8.44	9.18	8.27	8.37	8.09	1.16	0.17	0.80	0.61	0.27
Total period	6.70[a]	6.48[abc]	6.58[ab]	6.39[abc]	6.20[bc]	6.03[c]	0.45	**0.01**	0.44	0.64	0.22
CP *(kg/kg growth)*											
Day 0 - 84	0.72[a]	0.82[b]	0.91[c]	0.71[a]	0.74[ad]	0.80[bd]	0.09	**0.00**	**0.00**	0.07	0.34
Day 85 - 168	0.86[ab]	1.07[c]	1.21[d]	0.79[a]	0.93[be]	1.02[ce]	0.17	**0.00**	**0.00**	0.49	**0.05**
Day 169 - end	1.10[a]	1.33[b]	1.63[c]	1.02[a]	1.23[ab]	1.34[b]	0.26	**0.01**	**0.00**	0.45	0.32
Total period	0.85[ab]	1.03[c]	1.17[d]	0.80[a]	0.91[b]	0.99[c]	0.14	**0.00**	**0.00**	0.15	0.36
DVE[†] *(kg/kg growth)*											
Day 0 - 84	0.44[ab]	0.51[c]	0.62[d]	0.41[a]	0.46[b]	0.52[c]	0.08	**0.00**	**0.00**	0.07	0.30
Day 85 - 168	0.52[ab]	0.67[c]	0.83[d]	0.46[a]	0.57[b]	0.68[c]	0.14	**0.00**	**0.00**	0.40	**0.03**
Day 169 - end	0.67[ab]	0.83[c]	1.11[d]	0.60[a]	0.76[bc]	0.89[c]	0.20	**0.00**	**0.00**	0.31	0.15
Total period	0.52[a]	0.64[b]	0.79[c]	0.46[d]	0.56[a]	0.66[b]	0.11	**0.00**	**0.00**	0.10	0.10
NEF[†] *(MJ/kg growth)*											
Day 0 - 84	41.29[ab]	38.60[a]	38.19[a]	44.40[b]	41.16[ab]	39.36[a]	3.66	**0.01**	**0.00**	0.38	**0.03**
Day 85 - 168	49.10	49.72	50.62	50.06	50.55	50.34	4.42	0.87	0.77	0.92	0.29
Day 169 - end	63.39	62.15	67.60	65.53	67.26	64.63	8.29	0.70	0.81	0.57	0.63
Total period	48.96	47.72	48.61	49.86	49.79	48.54	2.90	0.43	0.86	0.63	0.89

[a,b,c,d,e]: means in a row with different superscripts are significantly different ($P < 0.05$)

[†] see footnote Table III.1.2

The significant effect of the factor year on the energy intake is probably caused by the differences in energy content of the maize silage between years. Each year, a different maize silage was fed, but all groups received the same silage within one year. The mean energy content of the maize silages for the three years equalled 6.9, 6.4 and 6.3 MJ NEF/kg DM respectively (Table III.1.2). As such, energy intake was higher for the first year, although DM intake was comparable (data not shown). The higher energy intake improved daily gain during the first year in comparison with the two other years and by consequence, live weight was also different between years. As mentioned before the animals of the first year were purchased at a higher LW, which caused an influence of the effect year on the initial LW (Table III.1.3).

Energy content exerted a similar effect on both intake and feed conversion (Table III.1.4 and III.1.5) when expressed as CP, DVE and NEF, while the results expressed as DM were less comparable. CP and DVE conversions were both significantly influenced by energy and protein level for the subperiods and for the total period. A high energy level improved the DM, CP and DVE conversion with 380 g, 120 g and 90 g per kg growth. Increasing the protein content negatively influenced the feed conversion when expressed as CP or DVE. This means that the groups fed the LP level had the best protein efficiency.

The different protein levels influenced the protein intake for all the subperiods and the total period.

The crude protein intake over the total period was the only parameter on intake or conversion for which a significant interaction between energy and protein was found. In the tables the interactions between the effects year and protein and/or energy are not listed because very few significant interactions were found. Only for the final live weight and for the growth rate between day 85 and 168, a significant ($P < 0.05$) interaction between protein and year effect was found. No interactions between the effects year and energy and/or protein were found for intake or conversion parameters.

Carcass and meat quality

The effect of energy and protein level on the carcass characteristics is shown in Table III.1.6. A significant influence of the energy level on the fasting weight loss was found, as well as on the SEUROP conformation and fatness score and on percentage fat and bone in the carcass. The fasting weight loss of the HE group (2.2 %) was 12 % lower than that of the LE group (2.5 %). This difference is mainly due to a lower tract fill as a consequence of the lower feed intake of the HE group as mentioned in Table III.1.5.

The protein level significantly influenced cold carcass weight, dressing proportion, SEUROP conformation and percentage bone in the carcass. The higher cold carcass weight of the MP and HP groups is mainly a result of the influence of the protein on live weight at slaughter, as mentioned in Table III.1.4, and to a smaller extent the result of the influence of the protein level on the dressing proportion. When two separate variance analyses were done, once with slaughter weight and once with dressing percentage as a covariant, both parameters were significant and the protein effect on cold carcass weight remained in both cases significant. However, when one variance analysis was done with both covariants at the same time, the protein effect on the cold carcass weight disappeared. The differences in live weight gain and dressing

proportion explained approximately 2/3 and 1/3 of the difference in cold carcass weight, respectively.

The mean value for the dressing proportion of the HP groups was 69.1 % while the LP groups had a mean dressing proportion of 68.5 %. The mean conformation score for the LP and the HP groups was respectively 16.5 and 17.2, this difference being smaller than one subclass. However, when a variance analysis was done with cold carcass weight as a covariant, the significant protein influence on SEUROP conformation and percentage bone in the carcass disappeared. This means that protein has very little direct influence on carcass quality.

Protein or energy did not influence the surface area of the LT samples. However there was an influence of energy on the proportion of fat in the carcass (LE: 12.3 *versus* HE: 13.1 %) while the proportion of meat in the carcass only tended to be influenced by energy (P = 0.07).

The different energy and protein levels had very little influence on the quality of the meat. The energy level influenced the proportion of moisture and fat in the LT sample (Table III.1.7): higher fat and lower moisture contents were found for the HE groups. The somewhat higher CIE-Lab L*-value for the MP groups was the only significant difference caused by the protein level.

The combination of very few influences of protein and energy level on the one hand, and the numerous influences of the year effect on the other hand, indicate that the influence of protein and energy on meat quality is very limited. The important year influences on most of the carcass and meat parameters are probably due to the difference in slaughter weight between the three years. Since slaughtering depends on the market demand, the slaughter weight varied between years. As such the mean slaughter weight for the three years averaged 707, 683 and 687 kg respectively.

Only 1 parameter (cold carcass weight) showed a significant interaction between the factors energy and protein. This was a consequence of the same significant interaction that was found for final live weight (Table III.1.3). Furthermore, a significant interaction was found between the protein and the year effect concerning cold carcass weight, while the interaction between the effect energy and year was significant for the LT moisture and protein content. No significant interaction was found for any other carcass and meat quality parameters.

In Table III.1.8 results of the growth and carcass characteristics have been gathered to calculate the daily carcass growth and the daily accretion of meat in the carcass. Assuming that the proportion of cold carcass weight/live weight averages 66.5 % at a weight of 350 kg (De Campeneere, unpublished data) and taking the cold carcass weight at the end of the trial and the duration of the trial into account, mean daily carcass growth can be calculated. This parameter multiplied with the proportion of meat in the carcass gives mean daily meat accretion.

A significant effect of the protein level was found on the carcass growth and on the daily meat accretion. For both groups with the LP level these parameters had markedly decreased (680 g meat accretion per day) in comparison with the four other groups (on average 730 g meat accretion per day).

The results of the feed conversion expressed per kg carcass growth or meat accretion generally confirm the results of Table III.1.5. They clearly indicate that the

HE level has a positive influence on the DM, CP and DVE conversions. For example, the LE groups need 690 grams more DM and 170 grams more DVE for each kg meat accretion.

III.1.5 Discussion

It is well known that low dietary protein levels may reduce animal performance (Boucqué *et al.*, 1980a; Levy *et al.*, 1980). However, Anderson *et al.* (1988) found lower optimum protein levels than in this experiment. They recorded a reduced growth rate with bulls from different breeds (Angus, Hereford and Simmental crossbred) when the CP content in the diet was lower than 120 g/kg DM. No difference was found between 120 and 140 g CP/kg DM. Levy *et al.* (1980) also suggested lower CP levels than we did, but in agreement with our experiments they concluded for Israeli Friesian bulls that the optimal protein level can be decreased when feeding heavier animals. These authors concluded that 140 g CP/kg DM is optimal in high-energy diets (11.1 MJ metabolisable energy/kg DM) until a weight of 300 kg, and for heavier animals 120 g CP/kg DM should suffice. With Angus bulls Martin *et al.* (1978) couldn't justify CP levels higher than 110 g/kg DM, except during the first 8 weeks of fattening (220-290 kg) when protein levels of 140 or 150 g/kg DM could be recommended.

In this experiment the lower growth rate of the LP groups and the somewhat lower growth rate of the HEMP group on the one hand and the comparable growth rates of the other three groups on the other hand, suggest that the optimal DVE content in the ration is close to 100 g/kg DM (160 g CP/kg DM) for dm bulls below 500 kg. For the subsequent period up to slaughter, the lowest CP content, namely 77 g DVE/kg DM (125 g CP/kg DM) seems to be just sufficient. The optimal content for the first subperiod is somewhat higher than the minimum 140 g CP/kg DM proposed by Boucqué *et al.* (1984) for a corresponding weight range while the possibility to lower the protein content at the higher live weight was not recognised by these authors. This could be explained by the difference in diet and growth rate between the two trials. In this experiment animals grew on average 1.43 kg per day, while the animals of the above-mentioned authors were fed a complete dry ration and achieved a daily gain of 1.1 kg. Concerning protein it can be concluded that dm BB bulls require very high protein levels during the first phase of fattening. The possibility, as found by several other authors to lower the protein content from a certain live weight on, is also valid for the dm animals, but starting at a higher live weight. Because a small but positive influence of the protein content was found on the dressing proportion, a small decrease of these characteristics could be a consequence of lowering the protein from a weight of 500 kg onwards. In contrast with Fiems *et al.* (1995a) who found a partial compensation of the slower first-period growth for the lower protein group, no compensatory growth was recorded in this study.

Table III.1.6: Effect of energy (E) and protein (P) level and year (Y) on carcass characteristics

Energy level	LE			HE			Pooled	P-value			
Protein level	LP	MP	HP	LP	MP	HP	SD	E	P	ExP	Y
Fasting weight loss (%)	2.6[a]	2.5[a]	2.3[ab]	2.2[ab]	2.1[b]	2.3[ab]	0.8	**0.00**	0.72	0.24	0.13
Cold carcass weight (kg)	457[a]	472[bc]	466[b]	455[a]	467[b]	476[c]	21	0.70	**0.00**	**0.03**	**0.00**
Dressing proportion (%)	68.5	69.1	69.1	68.5	68.8	69.0	1.2	0.44	**0.01**	0.83	0.08
LT[§] surface (cm^2)	151	157	154	155	157	160	21	0.28	0.28	0.66	**0.00**
SEUROP-classification											
Conformation [H]	16.2[a]	16.8[b]	17.1[b]	16.8[b]	17.1[b]	17.3[b]	1.2	**0.02**	**0.00**	0.59	**0.01**
Fatness [I]	5.5[a]	5.4[a]	5.6[ab]	6.0[bc]	5.8[abc]	6.1[c]	0.9	**0.00**	0.29	0.94	**0.00**
Carcass composition (%)											
Meat	74.6[ab]	74.6[ab]	75.0[a]	74.0[b]	74.7[b]	74.4[ab]	1.7	0.07	0.32	0.42	0.72
Fat	12.3[ab]	12.4[ab]	12.1[a]	13.2[b]	12.8[a]	13.3[b]	1.9	**0.00**	0.84	0.50	0.36
Bone	13.1[a]	13.0[ab]	12.9[ab]	12.9[ab]	12.6[bc]	12.4[c]	0.9	**0.00**	**0.05**	0.43	**0.00**

[a,b,c]: means in a row with different superscripts are significantly different ($P < 0.05$)

[H] S = 18, E = 15, U = 12, ..., P = 3 points

[I] Class 1 = 3 (very lean), Class 2 = 6, ..., Class 5 = 15 points (very fat)

[§] LT = *m. longissimus thoracis*

*Table III.1.7: Effect of energy (E) and protein (P) level and year (Y) on meat quality characteristics (*m. longissimus thoracis*)*

Energy level	LE			HE			Pooled	P-value			
Protein level	LP	MP	HP	LP	MP	HP	SD	E	P	ExP	Y
LT composition (%)											
Moisture	75.8[a]	75.7[a]	75.8[a]	75.6[ab]	75.7[ab]	75.5[b]	0.4	**0.00**	0.26	0.23	**0.00**
Protein	22.3	22.5	22.4	22.3	22.4	22.5	0.4	0.79	0.05	0.40	**0.00**
Fat	0.8[ab]	0.8[ab]	0.7[a]	0.9[ab]	0.9[b]	0.9[b]	0.3	**0.00**	0.76	0.59	**0.00**
Shear-force value (N)	49.3	49.3	50.9	47.6	47.4	46.6	11.2	0.08	0.83	0.90	0.47
Ultimate pH	5.59[a]	5.63[ab]	5.64[b]	5.62[ab]	5.60[ab]	5.61[ab]	0.93	0.60	0.39	0.08	**0.01**
Loose water value (cm^2)	4.34	4.23	4.20	4.25	4.31	4.27	0.68	0.79	0.86	0.66	**0.00**
CIE-Lab colour											
L*-value	40.8[ab]	41.5[ab]	40.1[a]	40.9[ab]	41.7[b]	40.6[ab]	3.10	0.48	**0.05**	0.87	**0.02**
a*-value	16.52	16.51	16.48	16.92	16.46	16.51	1.64	0.59	0.58	0.55	**0.00**
b*-value	13.74	13.96	13.63	14.27	14.18	14.01	1.71	0.08	0.54	0.80	**0.00**

[a,b,c]: means in a row with different superscripts are significantly different ($P < 0.05$)

Table III.1.8: Effect of energy (E) and protein (P) level and year (Y) on the calculated daily carcass growth and meat accretion and on feed conversion expressed per kg carcass and meat accretion (accr.)

Energy level	LE			HE			Pooled	P-value			
Protein level	LP	MP	HP	LP	MP	HP	SD	E	P	ExP	Y
Carcass growth (kg/day)	0.92[a]	0.99[b]	0.97[ab]	0.91[a]	0.96[ab]	1.01[b]	0.14	0.95	**0.00**	0.30	**0.00**
Meat accretion (kg/day)	0.68[a]	0.74[b]	0.72[ab]	0.68[a]	0.72[ab]	0.75[b]	0.10	0.88	**0.00**	0.35	**0.00**
Kg DM/kg carcass	9.95[a]	9.41[ab]	9.50[abc]	9.41[abc]	9.02[bc]	8.73[c]	0.64	**0.01**	0.24	0.87	0.13
Kg CP/kg carcass	1.27[ab]	1.49[c]	1.68[d]	1.18[a]	1.32[b]	1.44[c]	0.10	**0.00**	**0.00**	0.22	0.21
Kg DVE/kg carcass	0.77[a]	0.93[b]	1.14[d]	0.69[c]	0.82[a]	0.95[b]	0.06	**0.00**	**0.00**	0.18	0.05
MJ NEF/kg carcass	10.58	10.09	10.17	10.90	10.52	10.15	0.68	0.58	0.56	0.89	0.73
Kg DM/kg meat accr.	13.34[a]	12.61[ab]	12.66[abc]	12.72[abc]	12.09[bc]	11.74[c]	0.90	**0.03**	0.22	0.89	0.16
Kg CP/kg meat accr.	1.71[ab]	2.00[c]	2.24[d]	1.59[a]	1.77[b]	1.93[c]	0.14	**0.00**	**0.00**	0.23	0.24
Kg DVE/kg meat accr.	1.04[a]	1.25[b]	1.52[d]	0.93[c]	1.10[a]	1.28[b]	0.09	**0.00**	**0.00**	0.23	0.07
MJ NEF/kg meat accr.	14.18	13.52	13.57	14.74	14.09	13.64	0.96	0.51	0.50	0.94	0.75

[a,b,c,d]: means in a row with different superscripts are significantly different ($P < 0.05$)

Andersen (1978) states that energy is the main factor determining growth and carcass gain. In this study no significant differences between the two energy levels were found and the correlation between daily gain and energy intake over the total period was very low ($r = 0.16$). This suggests that an optimum energy level for growth is reached at 7.38 MJ NEF/kg DM. This was earlier confirmed by Fiems *et al.* (1990) with BB dm bulls, where different energy levels were induced by incorporation of fat. They did not found any influence of different energy levels, between 7.2 and 7.9 MJ NEF/kg DM, on growth rate. Zinn (1989) states that fat supplementation only promotes daily gain when energy level is insufficient.

In accordance with Fiems *et al.* (1990), who found no further improvement of the dressing proportion for energy levels higher than 7.42 MJ/kg DM, this study showed no effect of energy on dressing proportion. The percentage fat in the carcass was significantly influenced by the energy level, which is not in agreement with the above-mentioned authors, but in accordance with Prior *et al.* (1977).

Based on the improved conversion data, the slightly better conformation and the smaller fasting weight loss of the HE groups, an energy level of 8 MJ/kg DM could be recommended for the total finishing period.

Surprisingly, the results of the LEMP groups are, although not significant, systematically better than those of the LEHP group, while within the HE groups HP performed better than the MP group. Although this would suggest an interaction between protein and energy, only a significant interaction ($P < 0.05$) was found concerning live weight at the end of the trial. Probably, the animals who received the HE level are capable of utilising the higher protein level, as did the HEHP group, while the LEHP group received enough protein for optimal growth, but they received insufficient energy to use that extra protein. Holzer *et al.* (1986) also found an increased rate of live weight gain during the first period of his trial due to supplementation of the low protein diet, and in agreement with our results the magnitude of this effect was considerably lower for the low energy diets.

The lower CP and DVE intake of the HE groups are partly due to the somewhat lower protein levels of the HE diets than the corresponding LE diets. The significant influences of the protein levels on both the CP and the DVE intake are mainly a combined consequence of the three different protein levels and the absence of influence of these levels on the DM intake.

Based on the results of this trial we could conclude that the protein content between 370 and 500 kg is very determining, while it is less important for heavier bulls when the energy becomes more important. This theory is confirmed by a stepwise multiple regression analysis with as dependent variable the daily live weight gain (dlwg) and as independent factors live weight (LW; kg) and the daily intake (di) of energy (E; MJ NEF), DM (kg), CP (kg), DVE (kg) and OEB (kg). Daily intake of CP was the first independent variable entering ($P < 0.001$) the regression for the first subperiod (± 370 - 500 kg) followed by daily intake of DVE ($P < 0.018$). For the second subperiod (± 500 - 625 kg) the daily intake of energy ($P < 0.001$) and the mean live

weight ($P < 0.001$) were the only variables entering the equation. While in the regression for the third subperiod (± 625 - 690 kg) the only significant dependent variable was daily intake of energy ($P < 0.001$). The three equations are respectively:

1° period:

g dlwg = 555 + 1738 x diCP - 1457 x diDVE $R^2 = 0.53$

2° period:

g dlwg = 1148 + 30 x diE - 3 x LW $R^2 = 0.67$

3° period:

g dlwg = -530 + 23 x diE $R^2 = 0.56$

For the total finishing period the regression equation, using the same procedure was:

g dlwg = 1080 - 3 x LW + 26 x diE + 208 x diCP $R^2 = 0.75$

III.1.6 Conclusion

When comparing the results of this trial with protein requirements for non-double-muscled bulls, we conclude that BB dm finishing bulls have higher protein requirements. According to this study a protein level of 100 g DVE per kg DM (160 g CP/kg DM) is advisable during a weight range of 370 to 500 kg, while 77 g DVE per kg DM (125 g CP/kg DM) seems to be sufficient during the rest of the finishing period. Although the high energy level had no significant influence on live weight gain, the high energy level of 8 MJ NEF/kg DM is proposed in order to improve the feed conversion and to maintain an excellent conformation.

III Feeding trials

III.2 Influence of phased energy and protein feeding on performance and carcass quality

Redrafted from:

"Phase-feeding to optimise performance and quality of Belgian Blue double-muscled bulls"

by:

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III.2 Influence of phased energy and protein feeding on performance and carcass quality

III.2.1 Abstract

In order to optimise performance and carcass quality of Belgian Blue double-muscled bulls four phased feeding regimens were evaluated. All diets were fed *ad libitum* and consisted of 65 % concentrates and 35 % maize silage on DM base. Total period was divided into three phases (ca. 360-460, 460-570 and 570-680 kg). All four groups received the same maize silage, but the different energy and protein densities were applied using different concentrates. During the three phases, the negative control group (NC) constantly received a ration with a low protein and a moderate energy density; the second group (DP) received with each phase rations with decreasing protein density while the energy density remained moderate; the third group (IE) received rations with increasing energy density at a constantly high protein density and the last group (DPIE) received rations which decreased in protein and increased in energy density.

The NC grew significantly slower during the first subperiod (1.37 vs. 1.62 kg/day for the other groups), resulting in a significantly longer total finishing period. During the third period IE had the slowest growth. The NC group needed 21 and 20 days more than the DP and DPIE groups respectively, to reach the same slaughter weight. The NC had the lowest DM-intake during the first subperiod, IE during the third. No differences were found concerning feed conversion for the total period, when expressed as DM or NEF (net energy for fattening). IE had the worst protein conversion while NC had the best, with the two other groups being intermediate.

The only important significant difference concerning carcass quality was the improved dressing proportion of the IE and DP groups. Although significant differences in the fat content of the *m. longissimus thoracis* were found, they were of minor importance.

These results proved that for Belgian Blue double-muscled bulls, protein density of the ration can be decreased importantly with increasing live weight. Few differences were found between feeding a constant moderate energy level or increasing the energy from a low to a high level, if both schemes are combined with a decreasing protein density.

III.2.2 Introduction

In recent decades the Belgian Blue (BB) breed has evolved from a dual-purpose breed to a pure beef breed (Hanset, 1996). In 1994, 62 % of the beef produced in Belgium was provided by the BB, whereas in 1981, this was only 46 % (Bouquiaux and Hellemans, 1996). Within the BB the double-muscling phenomenon is extremely pronounced. According to Hanset (1996) 80 % to 85 % of the BB population is double-muscled (dm).

The evolution from dual-purpose towards dm animals has had important consequences on their anatomy and physiology. The anatomical changes, such as improved dressing proportion, a higher proportion of meat in the carcass and a reduced fat content (Clinquart *et al*., 1994; Uytterhaegen *et al.*, 1994) suggest higher N-requirements. The physiological changes, such as improved feed conversion and presumably lower maintenance requirements (Hanset *et al.*, 1979; Geay *et al*., 1982; Fiems *et al.*, 1999b) seem to have improved feed efficiency.

However, Boucqué *et al*. (1984) found that the crude protein (CP) density in the diets for dm bulls should exceed 140 g per kg, while a content of 120 g per kg was found to be sufficient for conventional BB bulls. Fiems *et al*. (1998; see also Chapter III.1) evaluated with BB dm animals three protein densities within each of two energy densities and found that the protein requirements of dm animals exceed the protein standards for conventional cattle, especially during the first phase of finishing. They concluded that 100 g DVE (true protein digested in the small intestine) per kg DM (160 g CP per kg DM) is the optimum protein density from 370 to 500 kg live weight. During the rest of the fattening period no advantage was found in feeding protein densities higher than 77 g DVE per kg DM (125 g CP per kg DM).

The higher protein need during the beginning of fattening was also mentioned by Holzer *et al*. (1986), who investigated the effect of protein supplementation from 90 to 140 g CP per kg DM within two levels of energy. During the first period, protein supplementation had a positive influence on daily live weight gain. Levy *et al*. (1980) also suggested that the protein level for optimum performance can be decreased when feeding heavier animals. Martin *et al*. (1978) found a need for a higher dietary protein content with Angus bulls, but only during the first eight weeks of fattening.

The theory supporting the decreasing protein requirements with increasing live weight is based on the changing composition of the body. With advancing age, energy deposition in the form of fat increases more than in the form of protein (van Es, 1977). As such the protein and energy needs might change during the fattening period unless the efficiency of accretion changes.

Fiems *et al*. (1990) and Dufrasne *et al*. (1995) emphasised the need for a high energy content in the diet for dm bulls. This was confirmed by Fiems *et al*. (1998) who proposed, as an optimum energy density for dm bulls, 8 MJ NEF (net energy for fattening) per kg DM in order to improve feed conversion and fasting weight loss and to maintain an excellent conformation. Boucqué *et al*. (1980c) found significant positive correlations between energy intake and daily gain in conventional BB bulls.

Prior *et al*. (1977) found more effects of an increased energy level, such as increased marbling score, quality grade, fat thickness and yield grade in Angus-Hereford crossbreds, than in Charolais and Chianina steers.

This trial stems from that of Fiems *et al*. (1998), whose results suggested that protein and energy needs might vary with increasing live weight. Now, the effect of phase-feeding on animal performance and carcass quality is investigated. The

treatments aimed to find an optimum relation between the protein and energy intake of the animal and its needs for optimum performance and carcass quality. Optimising energy and protein intake can reduce feeding costs, can optimise performance and is beneficial to the environment as N-excretion can be reduced.

III.2.3 Material and methods

Animals and management

During two consecutive years, a total of 104 BB dm bulls were used to investigate the effects of adjusting the protein and energy content of the diet to the changing needs of finishing bulls. Effects on performance and on carcass quality were evaluated. The animals were selected from the market supply based on their age (younger than 13 months), weight (between 275 and 325 kg), health, the quality of the their legs and conformation. Live weight at purchase averaged 299 kg.

After an adaptation period of about 2 months with a diet based on maize silage, grass silage and concentrates, the animals were divided into four homogeneous groups, based on body weight (weighings on three consecutive days), growth rate during the adaptation period, body conformation, age, the weight/age ratio and the standard deviation of the mean growth and weight of each group. The total finishing period was divided into three weight ranges (ca. 360 - 460, 460 – 570 and 570 – 680 kg). The animals were confined in straw-bedded loose houses. At the start of the experiment animals were weighed on three consecutive days. In between, weight was determined once every four weeks and on two consecutive days at the end of each weight range. At slaughter, a third weighing, after a fasting period of 20 hours, determined fasted live weight. The trial averaged 244 days.

Feeding and feed characteristics

Once daily the bulls were fed *ad libitum* a diet consisting of 35 % maize silage and 65 % concentrate on DM base. Both components were fed at the same time but separately. Water was freely available. Intake of concentrate and maize silage was recorded daily for each pen. Each year, the 13 animals of one treatment were divided over two pens, one pen with 8 animals and one with 5. The first year, 3 animals of group 2 had to be removed due to hazardous accidents or injuries. To compensate that loss, the second year an extra pen of three animals took part in the trial for that group.

All four groups received the same maize silage, but the different energy and protein densities were applied using different concentrates. The first group (negative control; NC) received a diet with a low protein (LP) and a moderate energy (ME) density during the total period. The protein density of the diets of the second group (decreasing protein; DP) decreased while the energy density remained constant at the ME level. They respectively received a high (HP), a moderate (MP) and a low (LP) protein level, during the three respective subperiods. The third group (increasing energy; IE) received constantly diets with a HP density combined with during the first, the second and the third subperiod a low, moderate and high (LE, ME and HE) energy density respectively. The fourth group (decreasing protein and increasing energy; DPIE) was successively fed: HPLE, MPME and LPHE. The feeding scheme is illustrated in Figure III.2.1 and Table III.2.1.

Some rations, the combination of high NEF-contents with low DVE-contents, were impossible to formulate and therefore, DVEc was introduced. DVEc stands for DVE corrected for negative OEB (degraded protein balance) and is calculated as follows (if OEB is < 0): DVEc = DVE + (0.75 x 0.85 x OEB), with 0.75 the amino acid content in the microbial protein and 0.85 the digestibility of the microbial protein in the intestine. If OEB is positive, no correction is needed. The low protein contents in high energy diets were then realised through the use of negative OEB values.

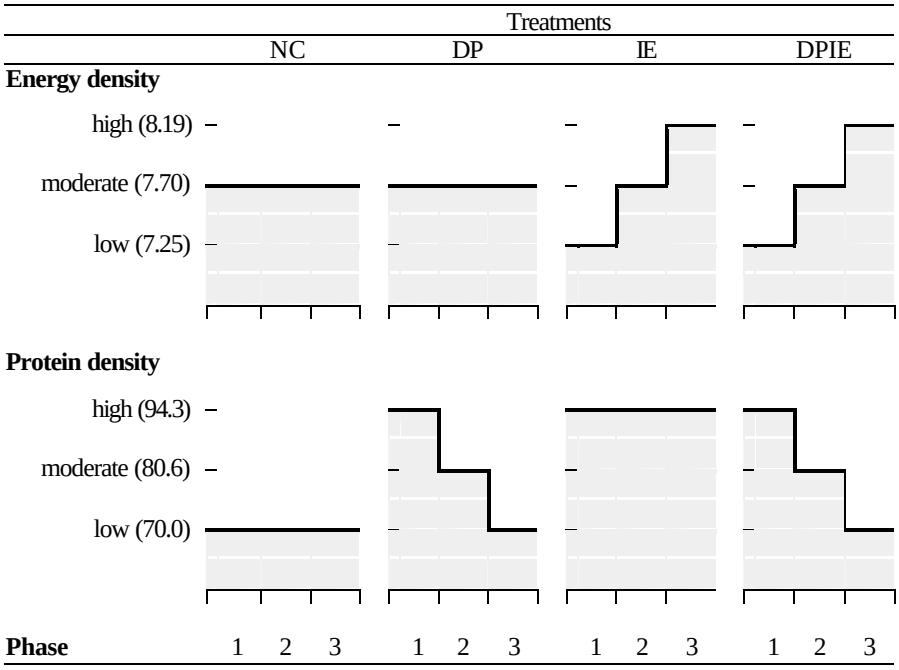

Figure III.2.1: Overview of the four treatments: protein (g DVEc/kg DM) and energy density (MJ/kg DM) of the rations in each of the three phases

The mean NEF densities of the LE, ME and HE diets were 7.25, 7.70 and 8.19 MJ per kg DM respectively, while the three protein densities averaged 70.0, 80.6 and 94.3 g DVEc per kg DM or 123, 143 and 156 g CP per kg DM respectively. The ingredients of the concentrates are shown in Table III.2.2. Chemical composition (Weende scheme) and nutritive value of the concentrates and the maize silages are shown in Table III.2.3. As the maize silage was different for each year, silage composition and values are listed per year. Energy values (NEF) were based on digestibility trials with wethers and calculated according to van Es (1978) while protein values (DVE and OEB) were determined according to Tamminga *et al.* (1994). For more details on the chemical analyses see III.1.3.

Table III.2.1: Feeding scheme of the trial with programmed (italic) and observed (bold) protein and energy values of the rations per subperiod.

Treatments							
NC		DP		IE		DPIE	
Diet Phase 1 (360 - 460 kg)							
LPME[†]		HPME		HPLE		HPLE	
DVEc[‡]	NEF[§]	DVEc	NEF	DVEc	NEF	DVEc	NEF
67	*7.42*	*93*	*7.42*	*93*	*6.90*	*93*	*6.90*
73	**7.61**	**96**	**7.66**	**103**	**7.25**	**103**	**7.25**
Diet Phase 2 (460 - 570 kg)							
LPME		MPME		HPME		MPME	
DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF
67	*7.42*	*80*	*7.42*	*93*	*7.42*	*80*	*7.42*
72	**7.71**	**81**	**7.79**	**94**	**7.74**	**81**	**7.79**
Diet Phase 3 (570 - 680 kg)							
LPME		LPME		HPHE		LPHE	
DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF
67	*7.42*	*67*	*7.42*	*93*	*7.94*	*67*	*7.94*
68	**7.71**	**68**	**7.71**	**84**	**8.13**	**68**	**8.26**

[†] L, M or H = low, moderate or high; combined with P or E = Protein or energy

[‡] DVEc = true protein digested in the small intestine corrected for a negative degraded protein balance (g/kg DM)

[§] NEF = net energy for fattening (MJ/kg DM)

Carcass quality

After a chilling period of 24 hours, the characteristics of the carcasses were determined. They were classified according to the SEUROP classification scheme (Anonymous, 1991a) and dressing proportion (cold carcass weight over fasted live weight) was calculated. Carcass composition (bone, fat and lean) was assessed by dissection of the 8th rib-cut (Verbeke and Van de Voorde, 1978), while the *m. longissimus thoracis* (LT) surface was estimated using a digitizer and a specific computer program to calculate the surface of the muscle starting from a picture of it.

To estimate the composition of the lean (moisture, fat and protein), a sample of the LT was taken at the 8th rib interface. Composition was estimated using NIRS (Near Infrared Reflection Spectroscopy) (De Boever *et al*., 1992).

Statistical analysis

Significance of the treatments was tested using the Univariate Analysis of Variance, with treatment and year as fixed factors. Differences between groups were based on the Duncan test ($P < 0.05$) (SPSS 8.0, 1998). The statistical units were individual data for live weight, live weight gain and carcass data, while pen data were the statistical units for intake and feed efficiency.

Table III.2.2: Ingredients of the concentrates (kg/tonne)

	Concentrates						
	LPME[†]	**HPME**	**HPLE**	**MPME**	**LPME**[‡]	**HPHE**[‡]	**LPHE**[‡]
Wheat	277	211	-	264	277	302.5	395
Tapioca	150	82	76.3	90	150	-	150
Malt sprouts	141	126.8	103	150	141	72	112
Sugar-beet pulp	138	-	290	-	138	50	44
Coconut meal	102	200	-	161	102	200	145
Beet molasses solubles	80	80	80	80	80	80	80
Pollards	61.6	138	150	134.5	61.6	-	-
Beef tallow	-	-	-	-	-	15.9	20.6
Maize glutenfeed	-	58	50	73	-	200	-
Rapeseed oilmeal	-	-	143	-	-	-	-
Protected soya-bean meal	-	56.5	68.4	-	-	31.8	-
Trace elements	15	15	15	15	15	15	15
Vitamin mix (A, D_3 and E)	7	7	7	7	14.5	14.5	14.5
Salt	2.7	2.4	2.1	2.5	2.7	2	2.7
Limestone	11.3	20.3	12.2	21.4	11.3	19.9	12.3
Feed phosphate	14.4	3	3	1.6	14.4	3.9	16.4
Total	1000	1000	1000	1000	1007.5	1007.5	1007.5

[†] L, M or H = low, moderate or high; combined with P or E = Protein or energy

[‡] the concentrates fed during the last subperiod were supplemented with 7.5 kg vit. E-premix per 1000 kg (i.e. 45 I.E. extra)

Table III.2.3: Chemical composition and nutritive value of the feeds and the rations

	Concentrates						Maize silage	
	LPME	HPME	HPLE	MPME	HPHE	LPHE	Year 1	Year 2
Chemical composition								
Dry matter (g/kg)	866	866	856	875	873	872	348	291
Composition of DM (g/kg DM)								
Crude protein	144	193	205	173	181	135	75	99
Ether extract	24	38	19	36	52	49	33	27
Crude fibre	92	85	141	78	83	72	186	193
N free extract	654	598	541	629	600	662	664	633
Ash	86	87	95	84	84	82	41	48
Nutritive value								
DVE[H] (g/kg DM)	88	114	125	92	97	85	58	63
OEB[I] (g/kg DM)	-2	24	18	23	28	-5	-36	-24
NEF[§] (MJ/kg DM)	7.94	7.98	7.32	8.10	8.62	8.82	7.19	7.16

	Rations					
	LPME	HPME	HPLE	MPME	HPHE	LPHE
Crude protein (g/kg DM)	124	156	164	143	148	118
DVE (g/kg DM)	78	95	103	81	84	76
OEB (g/kg DM)	-12	5	1	4	8	-13
DVEc[$] (g/kg DM)	71	95	103	81	84	68
NEF (MJ/kg DM)	7.77	7.70	7.27	7.77	8.12	8.25

Data are mean values of different analyses (one pooled sample per period per year, two experimental years)

[H] DVE = true protein digested in the small intestine

[I] OEB = degraded protein balance

[§] NEF = net energy for fattening

[$] DVE corrected for a negative OEB

III.2.4 Results

Animal performance

Table III.2.4 shows the effect of the treatments on duration of the trial, live weight and growth rate. The figures concerning live weight clearly indicate that each group changed to a subsequent feeding regimen when they exceeded the predetermined weight. The P-values of the influence of the treatment on live weight were all larger than 0.95 meaning that the groups changed diets at a comparable weight.

Table III.2.4: Influences of treatment (T) and year (Y) on live weight and growth rate

	Treatments				Pooled	P-value	
	NC	DP	IE	DPIE	SD	T	Y
Number of bulls	26	25	26	26			
Experimental days	256[a]	235[b]	250[ab]	236[b]	29	**0.01**	0.41
***Live weight** (kg)*							
Initial	360	360	361	361	25	0.99	**0.02**
460 kg	464	466	464	463	22	0.97	0.73
570 kg	569	570	571	573	26	0.96	0.42
Final	680	678	680	680	22	0.99	0.17
***Growth rate** (kg/day)*							
start – 460	1.37[a]	1.64[b]	1.63[b]	1.60[b]	0.21	**0.00**	**0.03**
460 – 570	1.38	1.52	1.47	1.48	0.20	0.29	0.05
570 – 680	1.08[ab]	1.11[ab]	0.96[a]	1.13[b]	0.23	0.06	**0.01**
Total period	1.26[a]	1.37[b]	1.29[ab]	1.37[b]	0.16	**0.02**	0.90

[a,b]: means in a row with different superscripts are significantly different ($P < 0.05$)

Significant differences were found concerning the duration of the trial. The NC needed 20 and 21 days more to reach the same slaughter weight as the DPIE and the DP groups. This was caused by a significant lower growth rate for the first period (1.37 *versus* 1.62 kg/day). The difference for the total period was mainly caused by the lower growth rate of the NC group during the first subperiod. During the second period no significant effect was recorded on daily gain. During the third subperiod the IE group grew slower than the other groups but only the difference with the DPIE group was significant. The initial live weight was different for the two replicates: 367 and 355 kg for the first and second year respectively. Growth rate during the first and third subperiod also differed between the two replicates. Both effects are very difficult to explain, but the former might possibly be caused by differences in management of the bulls before they arrive at the Department and the latter by climatic differences between the two years.

A significant ($P < 0.05$) interaction was found between the treatment and the year effect concerning the growth rate between the start and 460 kg. In the first trial the NC group grew 1.44 kg daily *vs*. 1.55 kg for the other groups, while in the second trial these figures amounted to 1.30 and 1.70 kg. The origin of this difference is very difficult to determine, although the variation in DM and CP content of the maize silages (Table III.2.3) might explain part of the difference. Also a possible difference in management of the bulls before arrival at the station, as mentioned above, may be at the origin of the difference.

The results of the feed intake are shown in Table III.2.5. The mean daily DM-intake over the first period of the NC group was 710 g or 9 % lower than the mean DM-intake of the other groups. This low DM-intake is probably caused by the low protein (*i.e.* the low OEB-level (degraded protein balance)) content fed to that particular group. For the second subperiod no differences in feed intake due to the treatments were found. For the third period on the contrary, the two groups with a high energy content ate on average 660 g DM less than the other groups.

Table III.2.5: Influence of treatment (T) and year (Y) on daily intake

	Treatments				Pooled	P-value	
	NC	DP	IE	DPIE	SD	T	Y
***DM** (kg)*							
start – 460	7.19[a]	7.78[b]	7.95[b]	7.96[b]	0.33	**0.01**	0.10
460 – 570	9.00	9.37	9.16	9.28	0.49	0.13	**0.00**
570 – 680	8.75[a]	8.91[a]	7.95[b]	8.39[b]	0.32	**0.00**	**0.04**
Total period	8.36[a]	8.74[b]	8.31[a]	8.56[ab]	0.25	**0.04**	0.11
***CP** (kg)*							
start – 460	0.91[a]	1.23[b]	1.30[b]	1.30[b]	0.04	**0.00**	0.65
460 – 570	1.12[a]	1.34[bc]	1.41[c]	1.32[b]	0.04	**0.00**	0.53
570 – 680	1.05[a]	1.07[a]	1.17[b]	1.00[c]	0.08	**0.00**	**0.00**
Total period	1.03[a]	1.20[b]	1.27[c]	1.18[b]	0.04	**0.00**	**0.04**
***DVEc†** (kg)*							
start – 460	0.52[a]	0.74[b]	0.81[c]	0.82[c]	0.03	**0.00**	0.64
460 – 570	0.64[a]	0.76[b]	0.86[c]	0.75[b]	0.04	**0.00**	**0.00**
570 – 680	0.59[a]	0.61[a]	0.67[b]	0.57[c]	0.04	**0.00**	**0.00**
Total period	0.59[a]	0.69[b]	0.76[c]	0.69[b]	0.02	**0.00**	0.17
***NEF†** (MJ)*							
start – 460	54.68[a]	59.58[b]	57.58[ab]	57.65[ab]	0.36	**0.04**	0.14
460 – 570	69.38[a]	72.97[b]	70.90[ab]	72.28[ab]	0.56	0.06	**0.00**
570 – 680	67.38[ab]	68.62[b]	64.55[a]	69.31[b]	0.39	**0.05**	**0.03**
Total period	64.20[a]	67.45[b]	64.62[ab]	67.10[ab]	0.29	**0.04**	0.13

[a,b,c]: means in a row with different superscripts are significantly different ($P < 0.05$)

† see footnote Table III.2.3

Especially the IE group had a very low DM-intake during that third period. For the total period the DM-intake of the DP group was highest, but not significantly different from that of the DPIE group.

A significant influence of the treatments on the daily intake of CP and DVEc was found for all three subperiods and for the total period. The NC group had the lowest daily protein intake, while the IE-group had the highest protein intake.

The energy intake during the first, third and total period was slightly influenced by the treatments. During the first and second subperiod energy intake of the NC group was lowest en during the third subperiod IE group had the lowest intake. The first differences were so important that for the total period the NC also had a lower energy intake than the DP group. During the second subperiod daily energy intake for the four groups averaged 71.4 MJ NEF at a growth level of about 1.46 kg per day. Several differences were found between the two replicates, especially during the second and third period.

Table III.2.6: Influence of treatment (T) and year (Y) on feed conversion

	Treatments				Pooled	P-value	
	NC	DP	IE	DPIE	SD	T	Y
***DM** (kg/kg growth)*							
start – 460	5.27^{a}	4.67^{b}	4.91^{ab}	4.95^{ab}	0.43	0.21	**0.01**
460 – 570	6.58	6.36	6.30	6.35	0.62	0.57	**0.00**
570 – 680	8.06	8.18	8.21	7.52	0.88	0.54	0.12
Total period	6.67	6.42	6.50	6.30	0.41	0.44	0.09
***CP** (kg/kg growth)*							
start – 460	0.67^{a}	0.74^{b}	0.80^{b}	0.81^{b}	0.06	**0.00**	0.13
460 – 570	0.82^{a}	0.91^{bc}	0.97^{c}	0.91^{b}	0.06	**0.01**	**0.01**
570 - 680	0.96^{a}	0.98^{a}	1.21^{b}	0.89^{a}	0.13	**0.01**	**0.01**
Total period	0.82^{a}	0.88^{a}	1.00^{b}	0.87^{a}	0.04	**0.00**	0.57
***DVEc**† (kg/kg growth)*							
start – 460	0.38^{a}	0.45^{b}	0.50^{c}	0.51^{c}	0.04	**0.00**	**0.03**
460 – 570	0.47^{a}	0.51^{b}	0.59^{c}	0.51^{b}	0.05	**0.00**	**0.00**
570 - 680	0.55^{a}	0.56^{a}	0.69^{b}	0.51^{a}	0.09	**0.02**	**0.00**
Total period	0.47^{a}	0.51^{a}	0.60^{b}	0.51^{a}	0.03	**0.00**	0.96
***NEF**† (MJ/kg growth)*							
start – 460	40.04^{a}	35.76^{b}	35.55^{b}	35.83^{b}	3.04	0.09	**0.01**
460 – 570	50.74	49.57	48.74	49.43	4.92	0.64	**0.00**
570 – 680	62.13	62.96	66.69	62.13	7.08	0.71	0.10
Total period	51.22	49.57	50.53	49.36	3.27	0.71	0.11

^{a,b,c}: means in a row with different superscripts are significantly different ($P < 0.05$)

† see footnote Table III.2.3

Although there was no significant influence of the treatment, LP content (NC) had an unfavourable influence, on the DM and NEF efficiency during the first two periods (Table III.2.6). On the contrary, CP and DVEc efficiency were significantly improved when feeding LP compared to HP. For the total period a significant worse CP and DVEc efficiency was found for the IE group which received constantly a HP level. Especially in the third subperiod the protein conversion of that group is very unfavourable: being about 29 % higher than the mean of the three other groups. During the first period a significant interaction between treatment and year was found for DM, CP and DVEc conversion. This was probably caused by the higher mentioned interaction between treatment and year for growth rate during that same period.

Carcass quality

The effects of the feeding regimens on the carcass characteristics are shown in Table III.2.7. Very few significant differences have been found. A significant effect of the treatment or the year was not found either on the fasting weight loss, on the SEUROP conformation and fatness score or on the proportion of bone in the carcass and the proportion of protein in the LT.

Dressing proportion and proportion of fat and water in the lean tissue was significantly influenced by the treatments.

Table III.2.7: Influence of treatment (T) and year (Y) on carcass characteristics

	Treatments				Pooled	P-value	
	NC	DP	IE	DPIE	SD	T	Y
Fasting weight loss (%)	1.9	2.3	2.0	2.1	0.8	0.92	0.58
Cold carcass weight (kg)	454^{a}	458^{ab}	465^{b}	455^{ab}	17	0.11	**0.05**
Dressing proportion (%)	68.1^{a}	69.1^{b}	69.6^{c}	68.3^{ab}	1.3	**0.00**	0.17
SEUROP-classification							
Conformation †	16.8	16.9	17.2	16.6	1.2	0.43	0.99
Fatness ‡	5.7	5.4	5.6	5.7	0.9	0.75	0.13
Carcass composition (%)							
Meat	76.2	76.4	76.1	75.3	2.0	0.12	**0.00**
Fat	11.3	11.0	11.1	11.9	2.1	0.17	**0.00**
Bone	12.5	12.6	12.8	12.9	0.8	0.51	0.06
LT composition (%)							
Moisture	75.8^{a}	75.7^{ab}	75.6^{ab}	75.5^{b}	0.4	**0.04**	**0.00**
Protein	22.4	22.5	22.6	22.5	0.3	0.31	0.95
Fat	0.9^{a}	0.9^{a}	1.0^{a}	1.1^{b}	0.3	**0.01**	0.26

^{a,b,c}: means in a row with different superscripts are significantly different ($P < 0.05$)

† S = 18, E = 15, U = 12, ..., P = 3 points

‡ Class 1 = 3 (very lean), Class 2 = 6, ..., Class 5 = 15 points (very fat)

§ LT = *m. longissimus thoracis*

Cold carcass weight, proportion of lean and fat in the carcass and proportion of moisture in the LT were significantly influenced by the effect year.

The IE group had the highest cold carcass weight, but this was only significantly different from the carcass weight of the NC. As the slaughter weights of the four groups were comparable (Table III.2.3) these differences are mainly a result of the improved dressing proportion. The mean dressing proportion of the DP and IE groups was 69.4 % while the other two groups had a mean dressing proportion of 68.2 %. The mean conformation score for the four groups was 16.9.

Some changes were found in the composition of the LT, although these differences have only minor practical importance. The DPIE group showed an increase in fat content in comparison with the other groups. For the protein content of the LT a significant interaction between treatment and year was found. Since this parameter only varied between 22.4 and 22.6 %, the importance of this interaction is negligible.

In Table III.2.8 results of the growth and carcass characteristics have been gathered to calculate the daily carcass growth and the daily accretion of meat in the carcass. Assuming that the proportion of cold carcass weight/live weight averages 66.5 % at a weight of 350 kg (De Campeneere, unpublished data) and taking the cold carcass weight at the end of the trial and the duration of the trial into account, mean daily carcass growth can be calculated. This parameter multiplied with the proportion of meat in the carcass gives mean daily meat accretion.

Table III.2.8: Influence of treatment (T) and year (Y) on the calculated daily carcass growth and meat accretion and on feed conversion expressed per kg carcass and meat accretion (accr.)

	Treatments				Pooled	P-value	
	NC	DP	IE	DPIE	SD	T	Y
Carcass growth (kg/day)	0.84[a]	0.93[b]	0.90[b]	0.92[b]	0.12	**0.02**	0.61
Meat accretion (kg/day)	0.64[a]	0.71[b]	0.69[ab]	0.69[ab]	0.09	**0.03**	0.23
Kg DM/kg carcass	9.94	9.36	9.19	9.35	0.62	0.22	0.30
Kg CP/kg carcass	1.22[a]	1.28[a]	1.41[b]	1.29[a]	0.07	**0.02**	0.19
Kg DVEc/kg carcass	0.70[a]	0.74[a]	0.84[b]	0.76[a]	0.05	**0.00**	0.39
MJ NEF/kg carcass	11.06	10.46	10.36	10.61	0.71	0.38	0.34
Kg DM/kg meat accr.	13.05	12.25	12.08	12.42	0.80	0.22	0.72
Kg CP/kg meat accr.	1.60[a]	1.68[a]	1.85[b]	1.72[a]	0.11	**0.01**	**0.04**
Kg DVEc/kg meat accr.	0.92[a]	0.96[ab]	1.11[c]	1.01[b]	0.07	**0.00**	0.08
MJ NEF/kg meat accr.	14.52	13.70	13.61	14.11	0.93	0.36	0.77

[a,b,c]: means in a row with different superscripts are significantly different ($P < 0.05$)

A significant effect of the treatments was found on the carcass growth and on the daily meat accretion. Due to the low dressing proportion and the longer trial period,

daily carcass growth of the NC group is significantly smaller than that of the three other groups. However, the daily meat accretion of the NC was only significant different from the DP group. The animals of that group accreted somewhat more than 700 gram meat per day.

The results of the feed conversion expressed per kg carcass growth or meat accretion generally confirm the results of Table III.2.6. No differences were found for DM or for NEF. CP and DVEc conversion indicate that IE is less efficient than the other groups.

III.2.5 Discussion

Many authors previously confirmed that low dietary protein levels reduce animal performance (Boucqué *et al.*, 1980a; Cobic *et al.*, 1980; Levy *et al.*, 1980; Anderson *et al.*, 1988; Fiems *et al.*, 1998). In most cases 120 g CP per kg DM is sufficient for optimum performance. Although in our study the protein density of the first period fed to the NC group was 123 g CP per kg DM, the NC grew significantly slower than the other three groups. This is in agreement with Fiems *et al.* (1998) who concluded that for BB dm bulls 160 g CP is advised for optimum growth during the first period of finishing (350 to 500 kg). They also concluded that 125 g CP should suffice for the rest of the finishing period. This is confirmed in this study, as there were no significant differences concerning growth between NC and the other groups during the second and third period. The somewhat lower growth of the NC during the second subperiod could be explained by the live weight at the end of the first subperiod, which was not comparable for the two studies. Whereas Fiems *et al.* (1998) proposed a high protein level until 500 kg, protein content was decreased from 460 kg onwards in this study. The results probably confirm the weight range for extra protein feeding proposed by the first study.

Cobic *et al.* (1980) confirmed the possibility to lower the protein content in the diet in three weight ranges with Dutch Friesian and Simmental x Friesian bulls: 140 g CP per kg DM until 250 kg, 120 g CP until 350 kg and 100 g CP until 435 kg. Rohr *et al.* (1982) listed optimum protein densities for Friesian bulls with different live weight, from 145 g CP at 200 kg to 110 g CP per kg DM at 500 kg. Anderson *et al.* (1988) also found an improved gain between 310 and 560 kg with Simmental crossbred bulls, when feeding 120 instead of 100 g CP per kg DM. At a higher live weight no difference between the two treatments was found. All these results indicate that a high dietary protein level is very important at lower live weights, but once passed a certain weight, animals only need lower protein levels. The threshold weight, and the optimum protein contents vary among studies, though none of them suggested levels of 160 g CP per kg DM as did Fiems *et al.* (1998) and 158 g CP as in this trial. This confirms the important difference between BB dm bulls and non-dm bulls or bulls of other breeds.

No important influence of the energy density on the growth rate was found. In the first period IE and DPIE groups received both HPLE while DP group received HPME. No differences in growth were found between these groups concluding that growth does not improve when raising the energy level to more than 7.25 MJ NEF per kg DM. During the second period all groups received the same energy density and during the third, NC and DP groups received ME while IE and DPIE groups were fed

HE. The only difference in growth rate during that third period was found between DPIE and IE groups, which received both HE. Fiems *et al.* (1998) neither found any influence of energy level on growth rate. Andersen (1978) however stated that energy is the main factor determining the growth and carcass gain. As we could not find any significant influence of the energy density on these parameters one could conclude that the lowest energy density fed, 7.25 MJ NEF per kg DM, is sufficient for optimal growth. This was earlier confirmed by Fiems *et al.* (1990) with BB dm animals, where different energy levels were induced by incorporation of fat. They did not found any influence of different energy levels, between 7.2 and 7.9 MJ NEF/kg DM, on growth rate. Zinn (1989) stated that fat supplementation only promotes daily gain when energy level is insufficient.

During the first subperiod the group with the low protein level (NC) had a reduced DM-intake. It is well known that limited protein feeding can reduce rumen fermentation due to a shortage of nitrogen in the rumen (Merchen *et al.*, 1987). A slower fermentation causes a reduced digestibility and consequently reduces passage rate, which in turn reduces DM-intake.

Most of the observed differences in CP and DVEc intake are imposed by the experimental design. The NC consumed least and the IE consumed most protein.

The lower energy intake during the first subperiod of the NC is a consequence of the earlier mentioned decreased DM-intake. The last one was caused by a reduced protein content of the diet. The significant lower energy intake during the third period of the IE group compared to the DPIE group is hard to explain, as they received about the same energy level.

In agreement with Cobic *et al.* (1980) CP and DVEc efficiency of the animals receiving LP is always better than those receiving HP.

Dressing proportion of the IE groups was significantly better than that of the others. This difference is very hard to explain. It might be caused by the difference in DM-intake during the third period, which influences gut fill, which on his turn can have influenced dressing proportion slightly. However, this effect should be minimised by the fact that dressing proportion is based on fasted live weight. Fiems *et al.* (1990 and 1998) found no improvement of the dressing proportion with energy levels higher than 7.4 MJ per kg DM. In correspondence with Fiems *et al.* (1990) percentage fat in the carcass was not significantly influenced by the energy level but this is not in agreement with Prior *et al.* (1977).

The minor differences concerning LT composition show that during the third period, HE increased LT fat content, if dietary protein content was low. The increase in fat is compensated for by a decrease in moisture.

Overall, several significant influences of the effect year were found. Whereas some of these significances concerning intake and feed conversion are caused by the differences in maize silage or from the fact that the animals might have been treated differently from one year to another before arrival at the Department, the significances concerning carcass quality are less easy explainable. Mean slaughter weight was only 8 kg higher for the first than for the second year. Climatic differences during the weeks or days preceeding slaughter may be of importance.

In general, very few differences were found between the DP and the DPIE group. But when the performances and the feed conversions are expressed in kg carcass and kg meat (Table III.2.8), the DP group proves to be somewhat better than the DPIE group. Although in the third period (Table III.2.6) the results are slightly better for the DPIE than for the DP. Due to the high dressing proportion, the IE group has comparable performances as the DP and DPIE groups, when expressed in kg meat accretion, but the feed conversion is worse.

Based on the improved growth of the animals during the first period the high protein level of 100 g DVEc per kg DM is recommended. During the second subperiod, the growth of the NC is not significantly different from the other groups, though it is more than 0.1 kg per day lower. This could suggest that the first phase should be extended upto 500 kg as mentioned by Fiems *et al*. (1998) instead of 460 kg. This was confirmed when the growth rate was plotted against the number of days in trial for the four treatments. The NC group grew slower during the first 100 days. At that time they weighed about 500 kg.

The comparable growth rate during the second subperiod of the animals with MP and these with HP, and the better feed conversion of the first groups during the same period, confirm the possibility to lower the protein level in the diet. From 460 kg onwards DVEc can be decreased towards 81 g DVEc per kg DM and later on to the minimum level of 68 g DVEc per kg DM for the final period of fattening.

The energy level did not influence the growth rate in any of the three periods. During the first period, DVEc conversion of the DP group (ME) was significantly better than the one of the groups receiving a low energy level (IE and DPIE). ME also improved DM and CP conversion, but not significantly. In the second phase, all groups received ME and in the third phase very little differences were found between the groups fed ME in combination with LP (NC and DP) and the group fed HE in combination with LP (DPIE). Daily DM and protein intake of the DPIE group was significantly lower in that period, while growth rate tended to be somewhat better. This resulted in a tendency towards an improved DM and protein conversion in the third period for the DPIE group. On the other hand, carcass quality of the DP group was somewhat better. Therefore, the extra cost of feeding 8.25 MJ NEF compared to 7.71 MJ during the third period seems not justifiable.

III.2.6 Conclusion

As a conclusion the following combination could be recommended to be fed to BB dm bulls in an attempt to optimise performance and carcass quality. In a first period from 360 towards 460 kg, 100 g DVEc (160 g CP) per kg DM in combination with 7.75 MJ NEF per kg DM should be provided. In the second period from 460 to 570 kg, protein content can be decreased to 81 g DVEc (143 g CP) per kg DM, while energy level can be maintained. And finally, from 570 kg until slaughtering, protein can be further decreased towards 68 g DVEc (120 g CP) per kg DM while energy can still remain around 7.7 MJ NEF.

IV Body composition of Belgian Blue double-muscled bulls

General outline of Chapter IV

To determine energy and protein standards, not only data on the performances are important, also data on the composition of the body are essential. Particularly data on the composition of the gain over the considered weight range are indispensable. Unfortunately, the chemical body composition of BB dm bulls has never been analysed before. Besides, the BB dm bulls have proven to be very deviant. As such, compositional data of other breeds can not be used. Therefore, the evolution of the body composition had to be estimated.

Two *in vivo* estimation techniques, urinary creatinine excretion (UCE) and urea infusion, were applied on a total of 46 bulls, and for each bull on four different live weights. But, 18 bulls out of the 46 were slaughtered, homogenised and subsequently analysed shortly after applying both estimation techniques at one of the four live weights. In order to have a homogenous spread over a weight range between 300 and 700 kg, some bulls were slaughtered after the first (around 360 kg), some after the second (around 460 kg), some after the third (around 570 kg) and some after the fourth application (around 680 kg) of both techniques. In total, 151 UCE determinations and 147 urea infusions were performed.

The resulting data can be divided in three sets. The first set are the results of the 151 UCE and 147 urea infusions. The second set, which is a part of the first set, are the results of the 18 UCE determinations and urea infusions prior to slaughtering on the homogenised bulls. The third set consists of the compositional data of these 18 slaughtered bulls.

Chapter IV.1: "*In vivo* estimation of body composition" describes how we determined the best technique to estimate *in vivo* body composition. Therefore, two groups of data were used. On the one hand the data on the body composition of the 18 bulls that have been homogenised and analysed. On the other hand, the results of the estimation techniques performed on these 18 bulls prior to slaughter. Combining these data allowed us to determine equations predicting the composition of an animal from the results of one of the evaluated estimation techniques.

Based on the precision of the different equations, the best estimation technique was selected and afterwards used to determine the changes in the body composition of the BB dm bulls over the considered live weight range (300 to 700 kg). These calculations together with some more details on the composition of the BB dm bulls are given in **Chapter IV.2: "Analysis and discussion of the compositional data".**

Apart from the *in vivo* estimation techniques, two *post mortem* estimation techniques were also evaluated with the data of the 18 bulls. De Campeneere *et al.* (1999c) estimated body composition from the chemical composition of the non-carcass parts. De Campeneere *et al.* (1999b) evaluated the chemical and the tissue composition of the 8th rib as predictors of the carcass composition. These results are not presented in this study since they can not be used to derive the energy and protein standards.

IV Body composition of Belgian Blue double-muscled bulls

IV.1 *In vivo* estimation of body composition

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IV.1 *In vivo* estimation of body composition

IV.1.1 Abstract

Two *in vivo* estimation techniques (urinary creatinine excretion (UCE) and urea infusion) were performed on 18 Belgian Blue double-muscled bulls. Fasted live weight (fLW) at the time of determination ranged from 308 to 710 kg. To determine UCE, animals were confined in metabolic cages, to collect urine over a four-day period. From the creatinine concentration of the urine sample and the total collected volume, UCE was calculated. Four days after the end of the collection period, bulls were infused with a urea solution, providing 130 mg urea/kg fLW. Urea space (US) was determined from the difference in plasma urea concentration between samples taken before and 12, 18 and 24 minutes after mean infusion time. The day after infusion, the bulls were slaughtered and subsequently the chemical body composition was determined.

Partly due to the large fLW range, kg water and kg protein in the empty body were quite accurately predicted by UCE ($adjR^2$ = 0.94 and 0.97) and by US ($adjR^2$ = 0.89 and 0.91). The precision of the prediction of the empty body fat component (in kg) was less good (UCE: $adjR^2$ = 0.77; US: $adjR^2$ = 0.70). However, the empty body composition of the bulls varied only slightly and linearly with increasing live weight so that the fLW was the best parameter to estimate kg empty body water and protein with both an $adjR^2$-value of 0.99. The empty body fat was estimated from that same parameter with an $adjR^2$ = 0.75. The equations predicting relative body composition from UCE and US had much smaller $adjR^2$-values (UCE: 0.50 for % EBWa, 0.40 for % EBF and 0.26 for % EBP; US: 0.54 for % EBWa, 0.41 for % EBF and 0.32 for % EBP). However, the RSD (residual standard deviation) and CV (coefficient of variation) were also much smaller, meaning that the predictions were better. Based on a comparison of the CV, it was concluded that the prediction of the relative composition from UCE and US was always slightly better than from fLW. But the improvements of both estimation techniques compared to fLW were never important enough to justify the work of the determinations. Therefore, it is concluded that due to the very slight changes in the body composition of the BB dm bulls and the large LW range, UCE or US could not significantly improve the estimation of body composition from fLW.

From the comparison of the results with other studies, it can be concluded that the double-muscled bulls have an extremely high urinary creatinine excretion, which can not entirely be explained by their increased protein content. As a second conclusion, time for equilibration of the urea in the water compartment was in this study found to be remarkably longer than in others, presumably due to the lower capillary density of the Belgian Blue double-muscled animals.

IV.1.2 Introduction

Compositional data

At slaughter, a lot of animal tissues are considered as offal, with fat being an important part of it. From an economic point of view reduction of total fat in the body and in the carcass is very important. Besides, as fat is found to be responsible for cardiovascular diseases, a reduction of fat would also be of interest for reasons of public health (Klurfeld, 1994; Reckless, 1987). Sinclair and O'Dea (1990) indicated that lean beef can be included in a cholesterol-lowering diet if the overall fat content of the diet is kept low. The only drawback of reducing fat content might be reduced flavour. Savell and Cross (1988) recommended a minimum fat content in meat products of 3 %.

As important differences in fat content exist between and within breeds, genetic selection is the first tool to select for leaner animals (Bass *et al*., 1990; Sinclair and O'Dea, 1990; Lamberson, 1994). Therefore, data on the chemical composition of different breeds are of interest.

The Belgian Blue double-muscled (BB dm) bulls largely dominate the Belgian beef market. Their extreme conformation and very lean carcasses are their major advantages. No data are available on the chemical body composition of these animals. In this study, 18 BB dm bulls with varying live weights were homogenised and analysed. Data about their body and carcass composition are discussed and related with the results of the *in vivo* estimation techniques.

***In vivo* estimation techniques**

Data on body or carcass composition are very important in nutritional experiments with meat-producing animals. The ideal technique would estimate body or carcass composition based on measurements on live animals. In recent years, several techniques that were originally developed for human medicine have been adapted for predicting *in vivo* composition of animals (De Campeneere *et al*., 2000a; Chapter II.3). For example, computerised tomography (CT scan; Young *et al*., 1999), nuclear magnetic resonance (NMR; Baulain, 1997) and dual-energy X-ray absorptiometry (Mitchell *et al.*, 1996) have proven their usefulness with smaller animals *e.g.* sheep and pigs. These techniques are very expensive, due to the high costs of the equipment involved. Moreover, they can't be applied to larger ruminants (cows, bulls etc.), due to the limited size of most apparates, as they were originally developed for human medicine. Therefore, less sophisticated techniques such as the ones based on UCE or urea infusion are feasible and realisable methods for *in vivo* estimation of body composition in larger ruminants.

Urinary creatinine excretion

Creatine is mainly formed in the liver and is transported by the plasma to the muscles where it is stored (Brody, 1994). Borsook and Dubnoff (1947) found that 98 % of the creatine reserves of the animal are present in the skeletal muscles, mainly in the form of phosphocreatine. From that creatine between 1.6 % (DelGiudice *et al*., 1995) and 2.8 % (Borsook and Dubnoff, 1947) is daily converted into creatinine, which is entirely (DelGiudice *et al.*, 1995) excreted in the urine. Dinning *et al*. (1949) concluded that daily excretion of creatinine is not affected by protein intake, but DelGiudice *et al.*

(1995) stated that dietary sources of creatinine or creatine (*e.g.* meat) may be important. The differences in excretion between individual animals are larger than the differences between the excretions from day to day in the same animal (Dinning *et al.*, 1949), and the lean tissue of an animal can be considered not to change markedly from day to day (Lofgreen and Garrett, 1954). Therefore creatinine excretion may be worthwhile in predicting differences in the lean tissue content of animals. Evidence has indeed been accumulated, showing that UCE was highly correlated to the lean tissue of the animal (Van Niekerk *et al.*, 1963; Forbes and Bruining, 1976; Schroeder, 1990).

To determine UCE a total urine collection over at least a 24 hour-period and a simple analytical method (colorimetric) is needed. In animal experiments, the urine is usually collected over several days and a composite sample is analysed. Except for the collection of the urine this technique is very simple, little time-consuming and requires no high investments.

In this study the adequacy of the UCE technique to estimate body composition of BB dm bulls was investigated.

Urea infusion

The dilution technique is based on a rather constant relationship between the empty body water volume and the other chemical components of the animal's body. If we can measure the amount of body water and body weight, the body composition can be estimated (Reid and Robb, 1971; Bartle and Preston, 1986).

Ideally, the marker used for infusion should not be toxic and should not have any physiological effect. It should diffuse rapidly and homogeneously over the total water compartment, it should not be metabolised and preferably not foreign to the body and there should be an accurate and convenient method to determine its concentration in the sample taken from the compartment (mostly a blood sample). The two most frequently used markers are urea and labelled water, either deuterium or tritiated water, but in the past also other markers have been evaluated, such as antipyrine and N-acetyl-1,4-aminoantipyrine (Topel and Kauffman, 1988) and Evans blue (for plasma volume; Wright and Russel, 1984a). The dilution technique has not only been applied with different markers, but also to several species: from cats (Kornberg *et al.*, 1952) over nursing foal (Geerken *et al.*, 1988) up to men (Bradbury, 1961).

Based on their study, Rule *et al.* (1986) concluded that before using any prediction equation for calculating body composition of cattle from *in vivo* measurement of dilution space, the equations should be tested with a sub-sample of the cattle population for which its use is intended. Accordingly, Hammond and Waldo (1985) decided that separate prediction equations might be required for different breeds. As BB dm bulls have proven to be anatomically and physiologically deviant from other breeds (Chapter II.1), it was important to establish separate prediction equations for these animals.

Therefore, in this study the adequacy of the urea infusion technique to estimate body composition of BB dm bulls was investigated.

IV.1.3 Material and methods

Experimental design

During two consecutive years, a total of 46 bulls were chosen out of 160 bulls, after a two-month adaptation period, to take part in an experiment involving a serial slaughtering procedure. They were purchased in the market at a live weight between 275 and 325 kg. The total experimental period was divided in three phases (*ca*. 360 – 460 kg, 460 – 570 kg and 570 – 680 kg). The animals were divided over four feeding regimens (NC, DP, IE and DPIE), differing in energy and protein content. All diets were fed *ad libitum*. The treatments were the same as the ones fed in the second feeding trial and were described in detail in Chapter III.2. In short, the negative control (NC) group (n = 10) constantly received a low protein level combined with a moderate energy level. With each phase, the protein level of the DP group (n = 12) decreased while a constant moderate energy level was fed. The energy level of the IE group increased (n = 12) in combination with a constant high protein level. The DPIE group (n = 12) received rations with increasing energy levels and decreasing protein levels. Only 10 bulls were allocated to the NC group, because no bulls of this group were slaughtered at the beginning of the experiment (see further).

At the beginning of each phase (± 360, ± 460 and ± 570 kg) and at the end of the experiment (± 680 kg) both estimation techniques were applied on all animals. Therefore, they were confined in metabolic cages to determine UCE and four days after the end of the UCE determination, the bulls were infused with urea.

In the course of the experiment 18 of the 46 bulls were slaughtered at different LW, the day after the urea infusion at ± 360, ± 460, ± 570 or ± 680 kg. Subsequently, they were homogenised and analysed to determine the relationship between the results of both *in vivo* estimation techniques and body composition. Nine bulls were slaughtered during each year of the trial. The 18 bulls were selected out of the groups DP, IE and DPIE, based on their live weight (LW), in order to have a homogeneous spread of the LW over the investigated LW range. The first group was the negative control group and therefore no animals of that group were used for homogenisation.

A schematic overview of the different applications of the estimation techniques and the slaughterings for homogenisation is given in Table IV.1.1. After the first application of both estimation techniques at the beginning of the experiment (± 360 kg), two animals from the groups DP, IE and DPIE were slaughtered over the two year period (animal 6, 12, 18, 29, 35 and 41 in Table IV.1.1). Their LW at slaughter ranged from 309 to 405 kg. After the second as well as after the third application of the estimation techniques (± 460 and 570 kg respectively), one animal from each of the groups DP, IE and DPIE was slaughtered. LW varied from 426 to 486 and from 543 to 593 kg for the animals slaughtered after the second and the third application respectively (animals 7, 13, 42 and 19, 30 and 36 respectively in Table IV.1.1). Finally, at the end of the trial, again two animals from the groups DP, IE and DPIE were homogenised, with LW ranging between 628 and 723 (animals 8, 14, 20, 31, 37 and 43 in Table IV.1.1).

Urinary creatinine excretion determination

To determine UCE, the bulls were confined in metabolic cages for a seven-day period. During that period they were fed *ad libitum* the same diet they were given during the preceding period. As such, during the first and second collection period they were fed the diet of the first phase, for the third and fourth period they received the ration of the second and third period respectively. For more information on the rations see Chapter III.2. Before entering the metabolic cages, the animals were shaved and washed to avoid pollution of the collected urine, and weighed during three consecutive days. During the first three days the bulls were accustomed to the metabolic cages. During the remaining four days total urine and faeces were collected separately. Urine was collected and weighed once daily and a representative sample was taken. Before the beginning of the collection, diluted sulphuric acid was added to the urine container to reduce pH below 3 and to prevent bacterial destruction of creatinine. Urine samples were stored in plastic bottles and frozen at –20°C until analysis for creatinine. Creatinine concentration in the urine was determined using a test combination based on an enzymatic photometrical procedure (Boehringer Mannheim, Belgium). UCE was calculated by multiplying the urinary concentration by the total urine volume.

Four days after the end of the collection period, a blood sample was taken from the jugular vein to determine blood creatinine concentration using the same colorimetric procedure.

Urea infusion

Four days after the end of the urine collection period in the cages, urea infusion was performed. Therefore, the animals were deprived from feed and water during a 16-hour period, and fasted live weight (fLW) was determined after that period. A blood sample was taken to determine plasma urea-N (PUN) before infusion (on this sample also the blood creatinine concentration was measured as referred to in the previous paragraph). Subsequently a solution containing 20 % urea w/v dissolved in 0.9 % saline was administered during a 2-min interval through a catheter, inserted in the jugular vein. The volume injected was determined to provide 130 mg urea/kg fLW. In order to know the exact volume of the infused solution, the syringes were weighed before and immediately after infusion. Blood samples were collected through the same catheter 12, 18 and 24 min after the mean infusion time. After infusion and after each sampling the catheter was flushed with saline, containing heparin, to avoid clotting. The blood samples were centrifuged and the plasma frozen for subsequent PUN analysis. Therefore, a photometrical Merckotest test combination based on the Berthelot Method (Merckotest 3334, Diagnostica Merck) was used. US was calculated for the three sampling times (US12, US18 and US24) using the following equation:

$$\text{US (\% of fLW)} = \frac{\text{mg urea-N infused}}{\text{change in PUN (mg/100 ml) x fLW (kg) x 10}}$$

To determine absolute US (l), fLW was removed from the equation.

Table IV.1.1: Overview of the applications of the estimation techniques and the slaughterings for homogenisation

	NC					DP						IE						DPIE					
Year 1																							
Bull	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
360 kg	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
						s						**s**						**s**					
460 kg	x	x	x	x	x		x	x	x	x	x		x	x	x	x	x		x	x	x	x	x
							s						**s**										
570 kg	x	x	x	x	x			x	x	x	x			x	x	x	x		x	x	x	x	x
																			s				
680 kg	x	x	x	x	x			x	x	x	x			x	x	x	x			x	x	x	x
								s						**s**						**s**			
Year 2																							
Bull	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
360 kg	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
						s						**s**						**s**					
460 kg	x	x	x	x	x		x	x	x	x	x		x	x	x	x	x		x	x	x	x	x
																			s				
570 kg	x	x	x	x	x		x	x	x	x	x		x	x	x	x	x			x	x	x	x
							s						**s**										
680 kg	x	x	x	x	x			x	x	x	x			x	x	x	x			x	x	x	x
								s						**s**						**s**			

x = application of both estimation techniques around the mentioned live weight
s = slaughtering and homogenisation following the application of the estimation techniques

Slaughtering procedure and chemical analysis

The 18 bulls were slaughtered in the experimental slaughterhouse of the Ghent University 5 km away from the Department, where all experiments were conducted. Bulls were not fasted before slaughter. After stunning, bulls were rapidly exsanguinated. During the slaughtering procedure all non-carcass parts (NCP) were separated from the carcass and gathered. All blood was collected and weighed separately. After bleeding, the head, feet and hide were removed, weighed and without any further treatment gathered with the NCP. After removal, the weights of the lungs and trachea, heart, liver, spleen, testicles and penis were recorded. All the organs were added to the NCP. Gut fill was assessed by weighing the gastrointestinal tract, before and after removal of its contents, and calculated by difference. The emptied gastrointestinal tract was added to the NCP. Empty body weight (EBW) was then determined as LW minus gut fill. Once the organs and the gastrointestinal tract were removed, the body was split in a right and a left half. All removable fatty tissues were weighed and added to the NCP. Finally the kidneys and the ears were removed and they completed the NCP. As such, the carcass was the remaining part of the body after removal of the total NCP, including all removable fatty tissues. The tail however was considered as a part of the carcass. At the end of the slaughtering procedure weight of the right carcass half (including half of the tail) was determined.

The right carcass half (CC) and the NCP were prepared for analysis according to the following procedure. As soon as possible after slaughtering, the CC and the NCP were separately frozen at –28°C. Therefore, the CC was divided into pieces with maximum dimensions of 60 cm. The deep-frozen CC and the NCP were separately homogenised and a sample of at least a tenth of the initial mass was taken. The samples were then autoclaved at 121°C during 6 hours. After cooling down, the samples were further homogenised in a meat cutter and again sampled. Once freeze-dried, they were ground and analysed. The weights of the different samples were recorded, at the different steps of the procedure, to correct for weight losses due to evaporation. Water, protein, fat, ash and energy in the CC as well as in the NCP were separately analysed. Dry matter was determined by weighing before and after lyofilisation. After grinding through a 1 mm sieve, the sample was than further dried in an oven at 103°C during 4 hours. Crude fat was determined according to EU method (Publication European Communities No. L15/29 (method B)) consisting of a hydrolysis with 3N HCl followed by a 6 hour Soxleth extraction (petroleumether). Crude protein and ash in the homogenised carcass and non-carcass components were determined as described above for the feed characteristics (see III.1.3). Gross energy content was determined using an iso-peribolic IKA-calorimeter C7000 (IKA, Heitersheim, Germany). Amino acid composition of the protein of the carcass and the non-carcass parts was determined, after hydrolysis according to Bech-Andersen *et al.* (1990), with an Eppendorf LC3000 amino acid analyser (Eppendorf, Hamburg, Germany). Before hydrolysis, the sample was oxidised with performic acid/hydrogen peroxide. Hydrolysis was performed using 6 N HCl containing 50 mg phenol. Amino acid separation was done with a cation exchanger and post column photometrical detection with ninhydrin. From a standard, tyrosine recovery was determined to be only 65 %. Results of analysis of tyrosine were therefore corrected to 100 %.

Water, protein, fat, ash, energy and amino acid composition of the protein in the empty body (EB) were calculated from the results of the CC and NCP composition. Lean body mass (LBM) was calculated as the EBW minus total fat weight.

Due to technical problems, body compositional data from one animal were unreliable and therefore all data concerning that animal were excluded from all statistical analyses. Consequently, the compositional data are based on 17 observations. After analysing the blood samples of the urea infusions, the results of one of the 17 remaining infusions were unacceptable (urea concentration did not increase after infusion) and were excluded from all statistical analyses. As such, the relations involving US are based on 16 observations.

Statistical analysis

Means, standard deviations, correlation coefficients and linear regressions were calculated using SPSS 8.0 (1998). To evaluate whether the different treatments had an influence on the composition of the bulls, covariance analyses was performed analysing the compositional data in function of the treatment with fLW as a covariant. Using linear regression, prediction equations were first calculated based on each of the three prediction parameters (UCE, US and fLW). Secondly, stepwise multiple linear regression was done to predict body composition from all available prediction parameters (UCE, urinary creatinine concentration, blood creatinine concentration, US12, US18, US24 and fLW).

IV.1.4 Results

It should be stressed once more that in the following part, only two sets of data were used. On the one hand the data on the body composition of the 17 bulls that have been successfully homogenised and analysed. On the other hand, the results of the estimation techniques performed on these 17 bulls (16 bulls for urea infusion) just prior to slaughter.

Compositional data

In Table IV.1.2, data on the FLW, as well as on EBW and on LBM of the 17 homogenised bulls are listed. The fLW of the 17 bulls varied between 308 and 710 kg, while EBW varied between 276 and 669 kg.

Table IV.1.2: Mean, standard deviation (SD) and range for the fasted live weight (fLW) the weight of the empty body (EBW) and the lean body mass (LBM)

	Mean	SD	Range
fLW (kg)	506.0	134.9	308.0 - 710.0
EBW (kg)	471.6	132.3	276.4 - 668.8
LBM (kg)	440.5	118.2	260.6 - 618.9
LBM (% of empty body)	93.7	1.8	90.3 – 96.5

Covariance analyses excluded any influence of the treatments on the composition. This is in agreement with the results of the parallel feeding trial (Chapter III.2), in which the same four rations were fed. The different treatments had no

influence on carcass composition (Table III.2.7). As such, all compositional data could be pooled for analysis.

Table IV.1.3: Mean, standard deviation (SD) and range for the weight and composition of the non-carcass parts, the right carcass half and the empty body

	Mean	SD	Range
Non-carcass parts weight (kg)	124.0	34.9	73.3 - 178.5
Non-carcass parts			
water (kg)	82.7	20.1	50.7 - 118.6
protein (kg)	24.9	7.6	13.4 - 35.3
fat (kg)	13.3	8.0	4.4 - 32.7
ash (kg)	4.3	1.2	2.4 - 7.2
energy (MJ)	1110	444	559 - 2068
water (%)	66.7	3.4	59.2 - 71.7
protein (%)	19.7	1.4	16.4 - 22.1
fat (%)	10.0	3.7	4.6 - 18.4
ash (%)	3.5	0.5	2.5 - 4.9
energy (MJ/kg)	8.6	1.4	6.5 - 11.6
Right carcass half weight (kg)	173.6	48.8	101.5 - 247.6
Right carcass half			
water (kg)	121.7	32.8	72.3 - 170.7
protein (kg)	35.3	10.2	20.3 - 49.7
fat (kg)	8.8	4.2	4.1 - 16.5
ash (kg)	6.9	2.4	3.5 - 10.6
energy (MJ)	1211	392	692 - 1844
water (%)	70.3	1.5	67.4 - 72.7
protein (%)	20.3	0.5	19.5 - 21.2
fat (%)	4.9	1.2	3.1 - 7.4
ash (%)	3.9	0.4	3.0 - 4.4
energy (MJ/kg)	6.9	0.5	6.1 - 7.9
Empty body weight (kg)	471.6	132.3	276.4 - 668.8
Empty body			
water (kg)	325.7	85.7	195.4 - 457.9
protein (kg)	95.2	27.9	54.0 - 134.7
fat (kg)	31.2	16.4	12.6 - 64.6
ash (kg)	17.6	6.0	10.1 - 26.8
energy (MJ)	3530	1233	1944 - 5722
water (%)	69.4	1.9	65.8 - 72.0
protein (%)	20.1	0.6	18.9 - 21.2
fat (%)	6.3	1.8	3.5 - 9.7
ash (%)	3.7	0.3	3.0 - 4.1
energy (MJ/kg)	7.4	0.7	6.4 - 8.7

In Table IV.1.3, data on the composition and the weight of the NCP, the CC and the EB of the 17 homogenised bulls are listed. On average, the carcass of a BB dm bull contained 70.3 % water, 20.3 % protein, 4.9 % fat and 3.9 % ash. For the empty body, the mean composition averaged 69.4 % water, 20.1 % protein, 6.3 % fat and 3.7 % ash.

Although the animals that were used for homogenisation received different rations and were slaughtered at different live weights, the composition of the empty body and the carcass varied only slightly. The results of the homogenisations showed very low fat contents in the dm animals, despite the higher LW of some of the homogenised bulls. In the carcasses, the fat content only varied between 3.1 and 7.4 %. As expected, the chemical fat content in the NCP was somewhat higher and ranged from 4.6 to 18.4 %. The maximum fat content in the EB was below 10 %, while the lowest fat content was 3.5 %. The protein content was very stable for the CC (between 19.5 and 21.2 %), but somewhat more variation was found in the NCP (between 16.4 and 22.1 %). As such, protein content in the EB only varied between 18.9 and 21.2 %. As the fat and the protein content were rather stable, the water content neither varied much. The water content in the EB ranged from 65.8 to 72.0 %. The ash content in the EB was always smaller than 4.2 and larger than 2.9 %.

Urinary creatinine excretion

In Table IV.1.4 data concerning the blood and urinary creatinine concentration, the daily urine production, the UCE (g/day) and the ratio UCE/fLW of the 17 determinations before homogenisation are listed. These figures are all based on a four day collection period, except blood creatinine concentration.

Table IV.1.4: Means, SD and ranges for different creatinine parameters of the 17 bulls

	Mean	SD	Range
Blood creatinine concentration (µmol/l)	199.2	43.5	130.0 - 279.7
Urinary creatinine concentration (mmol/l)	28.8	9.9	16.6 - 54.5
Daily urine production (l)	7.9	1.5	6.1 - 10.8
UCE (urinary creatinine excretion; g/d)	25.2	7.4	14.3 - 37.5
UCE/fasted live weight (mg/kg)	49.6	2.9	44.0 - 53.7

On average, the 17 bulls daily produced 7.9 litres urine, with a creatinine concentration of 28.8 mmol per litre, resulting in 25.2 grams urinary creatinine excreted daily. Taking the fLW into account, the mean urinary creatinine excretion per day averaged 49.6 mg per kg fLW. This parameter only varied between 44.0 and 53.7 mg/day.

The correlation coefficients between the different compositional parameters and the creatinine parameters are shown in Table IV.1.5. All relations in the upper part of the table were highly significant except those with urinary creatinine excretion per kg fLW. The latter was only significantly correlated with the daily creatinine excretion expressed in g/day. Empty body water (EBWa), empty body protein (EBP), fLW and LBM were mutually very highly correlated, while the correlations of these four parameters with empty body fat (EBF) were clearly less high. The blood creatinine

Table IV.1.5: Correlation coefficients between compositional data and different creatinine parameters (n = 17)

	EBWa (kg)	EBP (kg)	EBF (kg)	LBM (kg)	fLW (kg)	Blood creatinine concentration (µmol/l)	Urinary creatinine concentration (mmol/l)	Daily creatinine excretion (g/d)	Urinary creatinine / fLW (mg/kg)
EBP (kg)	0.99**								
EBF (kg)	0.83**	0.87**							
LBM (kg)	0.99**	0.99**	0.85**						
fLW (kg)	0.99**	0.99**	0.87**	0.99**					
Blood creatinine concentration	0.89**	0.87**	0.82**	0.89**	0.89**				
Urinary creatinine concentration	0.81**	0.81**	0.91**	0.81**	0.83**	0.86**			
Daily creatinine excretion	0.97**	0.99**	0.88**	0.98**	0.98**	0.87**	0.84**		
Urinary creatinine/kg fLW	0.41	0.48	0.46	0.43	0.44	0.33	0.46	0.59*	
	EBWa (%)	EBP (%)	EBF (%)	LBM (%)					
EBWa (%)					-0.69**	-0.61*	-0.74**	-0.73**	-0.50*
EBP (%)	-0.41				0.48	0.24	0.20	0.55*	0.70**
EBF (%)	-0.94**	0.17			0.63**	0.59*	0.79**	0.66**	0.39
LBM (%)	0.94**	-0.17	-1.00**		-0.63**	-0.59*	-0.79**	-0.66**	-0.39

*: P-value < 0.05; **: P-value < 0.01

concentration was higher correlated with EBWa, EBP, LBM and fLW than the urinary creatinine concentration. However, daily urinary creatinine excretion showed the highest correlations with all body components, except with EBF. The latter was best correlated with the concentration of creatinine in the urine. This indicates that total creatinine excretion will be the best predictor of the body composition, but if urine can not be collected over at least a 24-hour period, blood creatinine concentration is more appropriate than urinary creatinine to estimate the composition (except for the fat content). Based on this conclusion, regression equations will only be derived to estimate body composition from total urinary creatinine excretion (UCE). For the multiple regression analysis all three parameters will be used.

When body composition was expressed in percentages (bottom part of Table IV.1.5), most correlations decreased substantially. LBM (%) and EBF (%) are perfectly correlated (r = -1.0) as % LBM is calculated from EBF (kg) as follows: (EB (kg) – EBF (kg)) / EB (kg). For % EBWa, % EBF and % LBM the best correlations were found with urinary creatinine concentration. % EBP was best correlated with excreted creatinine in the urine per kg fLW.

In Table IV.1.6 the results of the regression analyses are listed. In the upper half of the table relations between UCE and absolute empty body composition of the 17 bulls are shown. EBWa and EBP were accurately estimated by UCE. EBP had the highest adjR^2 (0.97) and the lowest RSD (CV) value: 4.8 kg (5.0 %) *vs.* 21.4 kg (6.6 %) for EBWa. Estimation of the fat content was less successful, with an adjR^2 = 0.77 and a CV of 25.3 %. The LBM was also accurately predicted by the UCE.

Table IV.1.6: Empty body composition (kg and % of EB) and LBM (kg and % of EB) estimated from UCE (g/d) with adjR^2, RSD and CV (n = 17)

Prediction equation		adjR^2	RSD	CV (%)
kg EBWa	= 42.38 + (11.22 x UCE)	0.94	21.4	6.6
kg EBP	= 1.46 + (3.71 x UCE)	0.97	4.8	5.0
kg EBF	= -18.32 + (1.96 x UCE)	0.77	7.9	25.3
kg LBM	= 47.64 + (15.56 x UCE)	0.95	26.5	6.0
% EBWa	= 74.1 - (0.18 x UCE)	0.50	1.33	1.9
% EBP	= 18.9 + (0.05 x UCE)	0.26	0.54	2.7
% EBF	= 2.17 + (0.16 x UCE)	0.40	1.43	22.7
% LBM	= 97.8 - (0.16 x UCE)	0.40	1.43	1.5

The very high adjR^2-values, in the upper part of Table IV.1.6, are mainly due to the high variation in EBW. Therefore, body composition was expressed in percentages and the relations between the relative body composition and UCE were studied (lower half of Table IV.1.6). In comparison with the upper half of the table, the adjR^2-values were noticeably lower. The relation between % EBP and UCE was particularly weak (adjR^2 = 0.26). But, owing to the small compositional variation in this type of bull, the RSD values of the same equations were small, indicating that they give a good estimation of the composition.

After comparing the CV and RSD of the relative and absolute equations, it is concluded that the relative equations based on UCE gave the best fit to the body compositional data, although their R^2-value was remarkably lower.

Urea infusion

In Table IV.1.7 data on PUN (mmol/l) and US (l and % of EBW) (n = 16) at different times post mean infusion time are listed. Mean PUN increased from 3.73 mmol/l before infusion towards 8.03 mmol/l 12 minutes after the mean infusion time and again 12 minutes later it had decreased to 7.31 mmol/l. The corresponding US varied from 57.2 % to 68.3 % of the EBW at 12 and 24 minutes post mean infusion time respectively. Large variations were found in the absolute urea space (l), which was expected since the LW of the slaughtered animals is very divergent. The large variations in % US are more difficult to explain. At 12 minutes post mean infusion time, urea space varied between 44.3 and 74.4 % of the empty body. At T18 and T24, the range was 52.0 to 77.5 % and 55.0 to 83.9 % respectively, with the range at T18 being the smallest.

Table IV.1.7: Mean, SD and range for PUN at T0, T12, T18 and T24 and urea space (US; l and %) at T12, T18 and T24[†] (n = 16)

	Mean	SD	Range
PUN (mmol/l)			
T0	3.73	1.05	2.13 - 5.98
T12	8.03	1.31	6.08 - 10.71
T18	7.55	1.19	5.79 - 10.11
T24	7.31	1.17	5.56 - 9.82
US (l)			
T12	267.6	53.1	159.8 - 356.5
T18	301.2	56.1	200.0 - 364.8
T24	320.0	58.0	218.2 - 393.9
US (% of EBW)			
T12	57.2	8.7	44.3 - 74.4
T18	64.2	7.7	52.0 - 77.5
T24	68.3	7.7	55.0 - 83.9

[†]T0, T12, T18 and T24: before infusion, 12, 18 and 24 minutes after mean infusion time respectively

In Table IV.1.8 the equations predicting the weight of the EB components from US at three different times are listed. These relations are based on 16 observations. Kg EBWa and kg EBP were rather well estimated by US24; the determination coefficients were high (adjR^2 = 0.89 and 0.91 respectively). Unfortunately, the RSD and CV values were also rather large. Estimation of kg EBF was less successful, but the best results were obtained when blood was sampled at 18 minutes post mean infusion time.

The high adjR^2-values in Table IV.1.8 are mainly due to the large variation in EBW and the surprisingly small variation in body composition. Therefore, body

composition was expressed in percentages and the relations between the relative body composition and US were studied (Table IV.1.9). In comparison with Table IV.1.8, the adjR²-values were remarkably lower. Especially the correlation between % EBP and US was very weak, because protein was the most stable component. But, due to the very little compositional variation in this type of bulls, the RSD and CV values of the same equations were very small, which indicated that the equations predicted the relative body composition quite well. It is remarkable that the optimal sampling time is different for the prediction of the absolute and the relative composition. Kg EBWa and kg EBP were best predicted after 24 minutes, whereas % EBWa and % EBP were best predicted after 18 and 12 minutes respectively. Kg EBF and % EBF were both best correlated with US18.

After comparing the RSD and CV of the relative and absolute equations, it was concluded that the relative equations based on US gave the best estimation of EBWa and EBP. A smaller improvement was found in predicting EBF.

Table IV.1.8: Absolute empty body composition estimated from US12, US18 and US24[†] (l) (n = 16)

	Prediction equation	adjR²	RSD	CV(%)
EBWa	= -0.31 + (1.24 x US12)	0.58	54.5	16.7
(kg)	= -77.53 + (1.36 x US18)	0.81	37.2	11.4
	= -107.36 + (1.37 x US24)	**0.89**	**28.6**	**8.8**
EBP	= -16.49 + (0.43 x US12)	0.65	16.2	17.0
(kg)	= -40.28 + (0.46 x US18)	0.86	10.3	10.8
	= -47.38 + (0.45 x US24)	**0.91**	**8.5**	**8.9**
EBF	= -30.26 + (0.23 x US12)	0.52	11.5	36.9
(kg)	**= -43.75 + (0.25 x US18)**	**0.70**	**9.1**	**29.2**
	= -42.33 + (0.23 x US24)	0.63	10.1	32.4

[†]US12, US18 and US24 = urea space at 12, 18 and 24 minutes after mean infusion time

Table IV.1.9: Relative empty body composition estimated from US12, US18 and US24[†] (l) (n = 16)

	Prediction equation	adjR²	RSD	CV(%)
EBWa	= 76.2 - (0.026 x US12)	0.48	1.38	2.0
(%)	**= 77.1 - (0.026 x US18)**	**0.54**	**1.30**	**1.9**
	= 76.3 - (0.022 x US24)	0.40	1.48	2.1
EBP	**= 18.2 + (0.0072 x US12)**	**0.32**	**0.52**	**2.6**
(%)	= 18.2 + (0.0064 x US18)	0.27	0.54	2.7
	= 18.2 + (0.0059 x US24)	0.24	0.55	2.7
EBF	= 0.67 + (0.021 x US12)	0.31	1.58	25.1
(%)	**= -0.51 + (0.023 x US18)**	**0.41**	**1.45**	**23.0**
	= -0.08 + (0.020 x US24)	0.33	1.55	24.6

[†] US12, US18 and US24 = urea space at 12, 18 and 24 minutes after mean infusion time

Fasted live weight

From the results of the homogenisations (Table IV.1.3), the body composition seemed to vary only slightly and, more important, linearly within the LW range of this study. As such fLW could explain a major part of the variation in body composition that we found. Therefore, the use of fLW to estimate body composition was evaluated.

Table IV.1.10 shows the equations predicting EB composition from fLW, when the dependent variable is expressed in kg or in %. Very high correlations were found between the composition of the EB and the fLW when both elements were expressed in kg, with for water and protein an adjR^2-value of 0.99 and a RSD (CV) of 9.3 kg (2.9 %) and 2.7 kg (2.8 %) respectively. The fat content was less well correlated with fLW: adjR^2 = 0.75 and RSD (CV) of 8.3 kg (26.6 %).

The very high adjR^2-values are mainly due to the high variation in EBW and the surprisingly small variation in body composition. Therefore, body composition was expressed in percentages and the relations between the relative body composition and fLW were studied (lower half of Table IV.1.10). In comparison with the upper half of the table, the adjR^2-values were remarkably lower. Especially the correlation between % EBP and fLW was very weak, because protein was the most stable component. But, due to the very little compositional variation in this type of bulls, the RSD and CV values of the same equations were so small that the equations provided a good estimation of the composition.

After comparing the RSD and CV of the relative and absolute equations, the relative equations gave the best fit to EBWa, EBF, LBM and EBEn, while both type of equations were comparably accurate in predicting EBP and EBA.

The results of the stepwise multiple linear regression analyses are listed in Table IV.1.11. For these analyses not only the UCE, US and fLW were used, but also the concentration parameters of creatinine in the blood and urine. Estimation of kg and % EBWa, EBP and EBA and kg LBM did not improve significantly by combining different parameters. For each of these components, the equation resulting from the multiple regression analyses was the same as the best equation from Table IV.1.6 to IV.1.10 predicting that component. Surprisingly, urinary creatinine concentration was most accurate to predict % EBF, % LBM and MJ/kg EBW. It also improved the estimation of MJ EBEn from fLW and the estimation of kg EBF from US18.

Table IV.1.10: Empty body composition (kg and %), empty body energy (EBEn; MJ and MJ/kg) and LBM (kg and %) estimated from fLW (kg) (n = 17)

Unit		Prediction equation	adjR²	RSD	CV (%)
Kg	EBWa	= 5.80 + (0.632 (± 0.017) x fLW)	0.99	9.3	2.9
	EBP	= -9.07 + (0.206 (± 0.005) x fLW)	0.99	2.7	2.8
	EBF	= -22.68 + (0.106 (± 0.015) x fLW)	0.75	8.3	26.6
	EBA	= -4.11 + (0.043 (± 0.003) x fLW)	0.94	1.5	8.5
	LBM	= -1.60 + (0.874 (± 0.017) x fLW)	0.99	9.4	2.1
MJ	EBEn	= -951 +(8.86 (± 0.59) x fLW)	0.93	317	8.9
% of EBW	EBWa	= 74.24 - (0.0096 (± 0.003) x fLW)	0.44	1.41	2.0
	EBP	= 18.98 + (0.0022 (± 0.001) x fLW)	0.18	0.57	2.8
	EBF	= 1.93 + (0.0086 (± 0.003) x fLW)	0.36	1.47	23.3
	EBA	= 3.01 + (0.0013 (± 0.001) x fLW)	0.22	0.31	8.4
	LBM	= 98.07 - (0.0086 (± 0.003) x fLW)	0.36	1.47	1.6
MJ/kg EBW	EBEn	= 5.754 + (0.0032 (± 0.001) x fLW)	0.36	0.54	7.3

Table IV.1.11: Empty body composition (kg and %), empty body energy (EBEn; MJ and MJ/kg) and LBM (kg and %) estimated from fLW (kg), US12, US18 and US24 (l), UCE (g/d) and urinary and blood creatinine concentration (ucc and bcc; mmol/l)

Prediction equation		adjR²	RSD	CV (%)
kg EBWa	= 5.80 + (0.632 x fLW)	0.99	9.3	2.9
kg EBP	= -9.07 + (0.206 x fLW)	0.99	2.7	2.8
kg EBF	= -31.98 + (1.028 x ucc) + (0.113 x US18)	0.88	5.8	18.7
kg EBA	= -4.11 + (0.043 x fLW)	0.94	1.5	8.5
kg LBM	= -1.60 + (0.874 x fLW)	0.99	9.4	2.1
MJ EBEn	= -914.3 + (6.63 x fLW) + (37.6 x ucc)	0.96	242	6.9
% EBWa	= 77.1 - (0.0257 x US18)	0.54	1.30	1.9
% EBP	= 18.2 + (0.0072 x US12)	0.32	0.52	2.6
% EBF	= 2.01 + (0.1482 x ucc)	0.60	1.20	19.0
% EBA	= 3.01 + (0.0013 x fLW)	0.22	0.31	8.4
% LBM	= 98.0 - (0.1482 x ucc)	0.60	1.20	1.3
MJ/kg EBW	= 5.81 + (0.0541 x ucc)	0.58	0.45	6.1

IV.1.5 Discussion

Compositional data

The very low fat content and the small increase of that fat content with increasing LW were the most remarkable conclusions concerning the composition of the BB dm bulls. Due to the very low fat content, the LBM was proportionately very large.

The remarkably low chemical fat percentages in the CC (3.1 – 7.4 %) and in the EB (3.5 – 9.7 %) (Table IV.1.3) are in accordance with the low amounts of fatty tissue and intramuscular fat found by several authors in the BB dm bulls (Chapter II.1.). Ledger *et al.* (1973) found chemical fat contents in the carcasses of Boran and Hereford x Boran steers, with a slaughter weight between 137 and 448 kg, ranging from 6.3 to 40.0 %. Velazco *et al.* (1997) found with Holstein steers fat percentages in the carcass varying from 2.6 % at 100 kg towards 24.6 % at 465 kg body weight.

Figures IV.1.1 and IV.1.2 illustrate the changes in the absolute and relative empty body composition with increasing fLW as found with the 17 BB dm bulls. The water content decreases with 0.96 percent units for each 100 kg increase in fLW. This is mostly compensated for by a slight but linear increase in fat (0.86 percent units for each 100 kg fLW increase). The protein content in the empty body only increases very slightly (0.22 percent units for each 100 kg fLW increase). Although 17 homogenised bulls is a restricted sample to represent the total population, some important conclusions can be drawn. The BB dm bulls show very little variation in the proportions of the body components, at least for the considered weight range (Figure IV.1.2). Due to the hardly changed chemical composition at increasing slaughter weights, one could conclude that BB dm bulls are extremely late maturing animals. As such the carcasses and the meat of

these dm animals are still very lean at the conventional slaughter weights (650 – 725 kg).

The data on the body and carcass composition of the Belgian Blue dm bulls will be discussed more extensively in Chapter IV.2.

Urinary creatinine excretion

The daily UCE in this experiment varied between 14.3 and 37.5 g (Table IV.1.4). Regression analysis indicated that with each kg increase in fLW the daily excretion of creatinine increased with 54.1 mg (UCE (g/d) = -2.106 + 0.054*fLW; R^2 = 0.97). From the results of Schroeder (1990) this coefficient was derived to be 20.7 mg for a live weight range from 300 to 560 kg (UCE= 2.45 + 0.021*fLW). This large difference between the studies could partly be due to the higher protein accretion with each kg increase in fLW for the dm bulls.

The creatinine coefficients (Table IV.1.4) in this study (range: 44.0 – 53.7 mg creatinine/kg fLW) are extremely high in comparison with results from Lofgreen and Garret (1954): 24.3 – 37.3; Schroeder (1990): 24.71 – 29.22 and Dinning *et al.* (1949): 9.69 – 11.72. These three experiments were done with respectively Hereford yearling, crossbred (Simmental x Angus x Charolais; 300-560 kg) and Hereford steers (2 years old). The very low figures of Dinning *et al.* (1949) are quite deviant from these of Lofgreen and Garret (1954), although the same breed was used. The very high protein content in the empty body of the BB dm bulls in our experiment could have caused our high values. From our results, a mean excretion of 266 ± 0.012 mg creatinine for each kg protein in the empty body was calculated. From the results of Schroeder (1990), creatinine excretion was calculated at 300, 390, 480 and 560 kg live weight: 150, 176, 166 and 158 mg/kg protein respectively. Van Niekerk *et al.* (1963) found with 65 sheep of different breeds an average excretion of 195 g creatinine for each kg protein in the empty body. These figures prove that the high protein content in the dm bulls causes only part of the difference in the creatinine coefficient. This is in accordance with Hanset and Michaux (1982 and 1986), who found higher blood creatinine concentrations in dm than in non-dm bulls and concluded that the concentration in the dm type is higher than would be expected from the average hypertrophy of the muscles.

Whereas the high correlations (Table IV.1.5) between the UCE and the compositional data were expected, those between the urinary and blood creatinine concentration and the compositional data are somewhat surprising. They indicate that if collection of urine over several days is not possible, a simple blood or urine sample can also be used to predict the composition, with a blood sample being most precise in estimating EBWa, EBP and LBM, while a urine sample is better to predict the EBF.

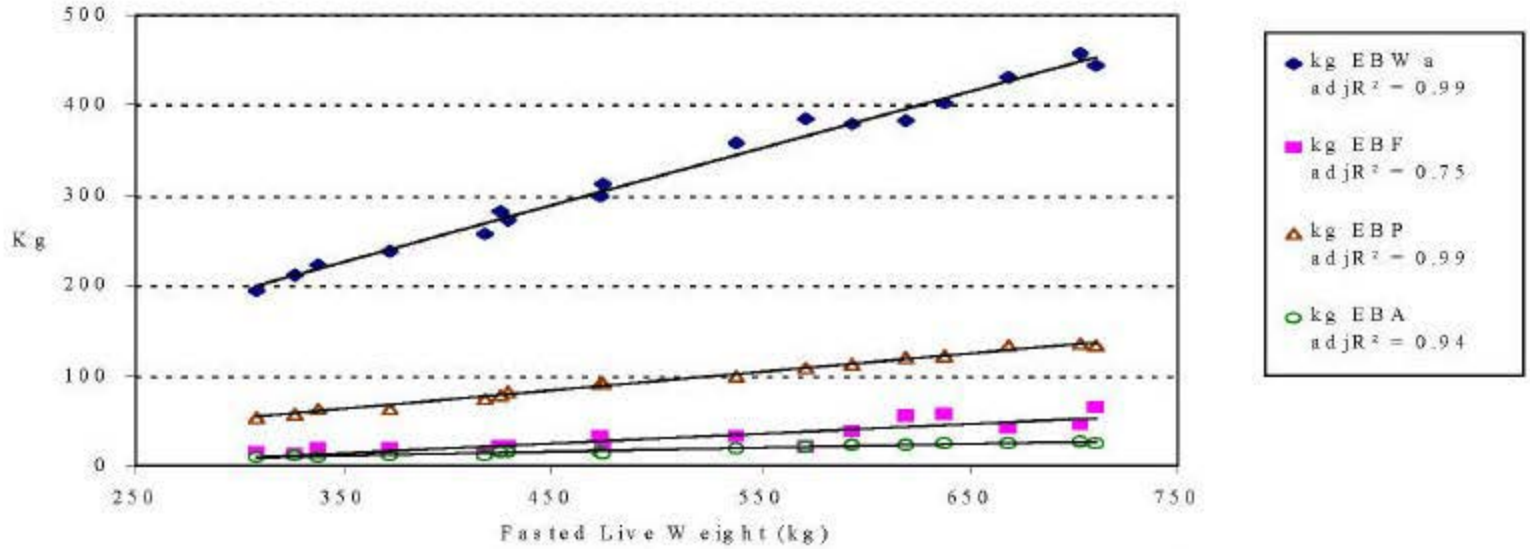

Figure IV.I.1: Absolute empty body composition of the homogenised bulls with increasing fasted live weight

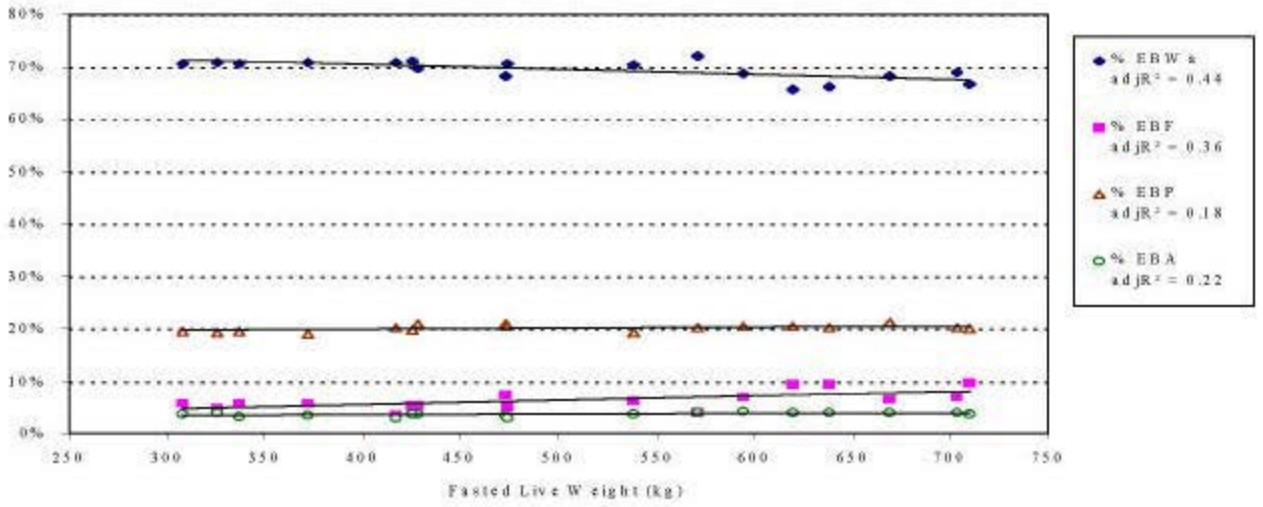

Figure IV.1.2: Proportional empty body composition of the homogenised bulls with increasing fasted live weight

Although the prediction of kg EBWa, kg EBP and kg LBM from UCE was reasonably good, it was even better when estimated from fLW (Table IV.1.10). The large range in body weight of the animals and the very small variation of the body composition, despite that large weight range and the different rations, are the most important reasons for this conclusion. Van Niekerk *et al*. (1963) made the only study on UCE in ruminants in which the precision of estimating EBP from a weight parameter (fLW) was mentioned. Both parameters were highly correlated (0.969) but UCE and EBP were still somewhat better correlated (0.972). Schroeder (1990) demonstrated (Table IV.1.12) that the addition of LW to the equation could significantly improve the precision of prediction of LBM (RSD from 22.2 to 6.63 kg) and EBP (RSD from 5.46 to 1.87 kg). In our study, no significant increase in precision was found by combining fLW and UCE, in predicting relative or absolute body composition.

In contrast to absolute weights of EBP, EBWa and LBM, that of absolute EBF was, in our study, somewhat better estimated from UCE than from fLW. But, the improvement was not large enough to justify the extra work and costs of the determinations.

Comparison of the Tables IV.1.6 and IV.1.10 indicate that the relative body composition is somewhat better predicted based on UCE than on fLW, but the differences are so small that both techniques could be considered equally precise and that the extra work of determining UCE can not be justified. It indicates however that the technique might be valuable for a population with more variation in body composition. From the analysis of the CV one can conclude that despite the very high adjR^2-values of the absolute predictions, the prediction of the relative body composition is somewhat more precise.

In Table IV.1.12 the results of a few studies that used UCE to predict body composition are given. The coefficients proposed in this study are always some 25 % lower than those of other studies with ruminants are. This is a consequence of the earlier mentioned higher creatinine concentration in dm cattle. No equations were found in literature predicting EBF.

Urea infusion

The equations predicting absolute body composition from US gave quite satisfying results for kg EBWa and kg EBP (adjR^2 = 0.89 and 0.91 and CV = 8.8 and 8.9 %, respectively). For the fat component however, the results were less successful: adjR^2 = 0.70 and CV = 29.2 %. In comparison with some results from other authors (Table II.3.3) such figures for water and protein are quite normal, while those for fat are actually rather good.

Table IV.1.12: Equations from literature predicting empty body components (kg) from UCE (g/d)

Authors	Based on UCE	R^2	Based on UCE and LW	R^2
	Empty body water			
Van Niekerk *et al.* (1963)				
65 sheep of different breed	= 3.62 + (15.56 x UCE)	0.94		
	Empty body protein		**Empty body protein**	
Schroeder (1990)				
20 Simmental x Angus x Charolais (300 - 560 kg)	= 13.64 + (4.92 x UCE)	0.81	= 13.26 + (0.81 x UCE) + (0.11 x LW)	0.98
	Lean body mass		**Lean body mass**	
Schroeder (1990)				
20 Simmental x Angus x Charolais (300 - 560 kg)	= 60.95 + (21.82 x UCE)	0.84	= 59.37 + (4.92 x UCE) + (0.45 x LW)	0.98
Van Niekerk *et al.* (1963)				
65 sheep of different breeds	= 4.50 + (21.12 x UCE)	0.94		
Forbes and Bruining (1976)				
21 men and 13 women	= 7.38 + (29.08 x UCE)	0.98		

The adjR^2 and RSD-values in Table IV.1.10 indicate very clearly that absolute body composition can be predicted much better by simply using fLW than by the results of the urea infusion. The high correlations found in this study between fLW and kg EBWa and kg EBP are quite exceptional. As mentioned before, the large range in body weight of the animals used in this study and the very small but linear variation of the body composition despite that large weight range are the most important reasons for that conclusion. Also in most other studies, a weight parameter (*e.g.* LW, fLW, EBW) could explain more of the variation than US could, especially when the variation in body weight was large and body components were expressed in kg. However, the correlations were rarely as high as in this study. Velazco *et al.* (1997) published equations, for Holstein steers between 3 and 12 months of age, predicting kg water and protein in the carcass from body weight with an R^2 = 0.97 and 0.96 respectively. With mixed-breed steers (210 to 517 kg) Hammond *et al.* (1988) found a R^2-value of 0.93 between EBW and kg EBP, which was slightly better than the 0.90 for the prediction based on US (Table II.3.3). The same researchers found for the same parameter with Angus steers (219 – 517 kg) a R^2 = 0.87, which again was somewhat better than if estimated from US (0.85) (Table II.3.3). Reid and Robb (1971) mentioned a very high correlation between the same parameters; R^2 = 0.997. These results were obtained with heifers ranging from 1 day to 14 months (± 45 to 240 kg).

The equations predicting relative (%) body composition from US and fLW (Tables IV.1.9 and IV.1.10) indicated that the % EBWa, % EBP and % EBF were only slightly better predicted from US than from fLW. However, the differences are so small that both techniques could be considered as equally precise in estimating relative composition and therefore, the extra work for the urea infusion can not be justified. It indicates however that the technique might be very valuable for a population with more variation in body composition. From the analysis of the CV one can conclude that despite the very high adjR^2-values of the absolute predictions, the predictions of the relative body composition from US and fLW were more precise.

One of the main problems to estimate EBWa in ruminants with urea infusion, is the differentiation of the gut water from the empty body water. According to Bartle and Preston (1986) urea only diffuses very slowly into reticulo-ruminal water and the quantity diffused during the 12 minutes after infusion is of no significant importance. This could explain why *e.g.* Preston and Kock (1973), who fasted their animals, and *e.g.* Bartle *et al.* (1987) who did not remove the feed before infusion, both found satisfying correlations. Bartle and Preston (1986) proved that urea concentration in the urine increased very quickly after infusion, indicating that urea diffuses in the urine pool. They concluded that urea determines empty body water and urine. Therefore it overestimates the EBWa with the volume of urine produced during the dilution time (Bartle and Preston, 1986).

In contrast with most publications on urea infusion (Kock and Preston, 1979), we found the highest correlation between kg EBWa and US at 24 minutes post mean infusion time (Table IV.1.8) instead of 12 minutes. However, % EBWa was best estimated from US18. Presumably, the urea was not entirely divided over the total water compartment at 12 minutes after mean infusion time. This is suggested by the coefficients of the US in the equations that predict kg EBWa. If urea was divided over

total body water (and urine in the bladder) one would expect this coefficient to be near 1 (and even somewhat less) and the constant in the equation to be rather small. In our studies the coefficient varied between 1.24 and 1.37 for the equations in Table IV.1.8, whereas in most other studies, it is close to and smaller than 1 (Table II.3.3). When regression analysis is done with exclusion of the constant, a coefficient close to 1 is definitely to be found when urea has equilibrated over the total body water. However, we found equations without intercept predicting kg EBWa from US12, US18 and US24 with respective coefficients 1.24, 1.11 and 1.05. The coefficient after 24 minutes is close to the coefficient proposed by Meissner *et al.* (1980) after 12 minutes, which suggests that the urea was almost divided at that time. Another indication that urea has not equilibrated after 12 minutes post mean infusion time is the difference in decrease of the PUN concentration between T12-T18 and T18-T24 (Table IV.1.7). If urea had been equilibrated after 12 minutes, both decreases should have been comparably large.

These results suggest that urea diffuses more slowly in BB dm bulls than in other breeds. One possible reason might be the lower capillary density of dm animals, so that the diffusion from blood into the tissues is slower. Stavaux *et al.* (1994) found significantly fewer capillaries per mm² in dm calves than in nc calves. Ashmore and Robinson (1969) noted, while doing a biopsy, a visual difference in bleeding between the same types of animals, with the dm calves bleeding less intense.

This includes that this technique might be less suitable for BB dm animals. The biggest advantage of urea infusion is that within a short equilibration time (less than 12 minutes) urea has diluted over the total body water volume and one single blood sample is than sufficient to estimate that volume. However, when urea diffuses more slowly in dm animals, the blood sample should be taken later, and within that extra time, urea also diffuses further into the urine pool and into the reticulo-ruminal water. Both factors will negatively influence the precision of the estimation. From our results we can not certify whether the urea had diffused completely after 24 minutes. To do so, blood samples should have been taken for a longer period, and regression analysis should indicate that from that time, PUN decreases linearly.

The multiple regression analyses resulted mostly not in a significant improvement of the estimation of the body composition. Prediction of kg and % EBF (and consequently % LBM and the energy component) could be improved when predicted from urinary creatinine concentration instead of US, UCE or fLW. This was expected from the correlation coefficient in Table IV.1.5, that indicated that EBF was best correlated with that concentration. However, this correlation is very difficult to explain since creatinine is physiologically a predictor of the protein component. Besides, it is even more surprising that the creatinine concentration is more accurate than the UCE, since creatinine concentration is much easier influenced than UCE.

IV.1.6 Conclusion

The composition of the BB dm bulls has revealed to be quite stable and to change only slightly and linearly with increasing LW. From the compositional data it is obvious that the BB dm bulls have a quite unique low-fat composition.

UCE revealed to be quite accurate in predicting absolute and relative EBWa, EBP and LBM, but it is less successful in estimating EBF. The estimation of the relative body composition from UCE is always somewhat more precise, than the estimation of the absolute composition. From the comparison of these results with other studies, it can be concluded that the dm bulls have an extreme high creatinine concentration, which can not entirely be explained by their increased protein content.

Just like UCE, urea infusion was quite accurate in predicting absolute and relative EBWa, EBP and LBM, but somewhat less successful in estimating EBF. The estimation of the relative body composition from US is always somewhat more precise, than the estimation of the absolute composition. In contrast with most other studies urea seemed not to have equilibrated at 12 min post mean infusion time. This could be a consequence of the lower capillary density of BB dm bulls. To confirm this further research has to be done. Due to the longer dilution time in dm animals, blood samples should be taken over a longer interval post infusion, to determine at which time urea has equilibrated.

Both techniques were positively evaluated. UCE was clearly more accurate to estimate absolute empty body composition, but fLW obviously excels both techniques in predicting EBWa, EBP and LBM. Although UCE is the best predictor for EBF, the difference with fLW is to small to justify the work of the UCE determination.

But, due to the large LW range and the small variation in body composition, no important differences were found concerning the accuracy of the prediction of relative body composition using UCE, US or fLW. The insignificant extra precision from using UCE or urea infusion in comparison with fLW was not large enough to justify the extra work of the infusion or the urine collection. Therefore, fLW was most useful to estimate the body composition.

Urea infusion and UCE might be useful in populations with more variation in composition and a smaller live weight range. For the purpose of this study, calculating protein and energy accretion, the fLW will be used.

IV Body composition of Belgian Blue double-muscled bulls

IV.2 Analysis and discussion of the compositional data

Redrafted from:

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De Campeneere, S., Fiems, L.O., De Paepe, M., Vanacker, J.M., Boucqué, Ch.V.

submitted to:

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IV.2 Analysis and discussion of the compositional data

IV.2.1 Abstract

Compositional data of 17 Belgian Blue double-muscled bulls with an empty body weight between 276 and 669 kg are discussed. Body composition only changed slightly and linearly within the investigated weight range. Water, protein, fat and ash in the empty body varied between 65.8 and 72.0 %, 18.9 and 21.2 %, 3.5 and 9.7 % and 3.0 and 4.1 % respectively. The chemical fat content in the empty body was remarkably low. The protein and fat accretion per kg growth varied from 197 to 211 g and from 73 to 130 g respectively between 350 and 700 kg live weight. Energy accretion varied from 7.57 to 9.68 MJ for the same weight range. The percentage of accreted energy as protein always remained higher than 50 % of the total accreted energy, which is much higher than generally reported in literature. From our dataset, caloric values for protein and fat were derived by regression: 22.91 ± 0.90 MJ/kg and 38.74 ± 1.53 MJ/kg respectively. Carcass protein contained on average (in %) 8.8 Asp, 15.6 Glu, 5.3 Pro, 7.7 Gly, 6.6 Ala, 7.5 Leu, 7.4 Lys and 6.5 Arg, while the protein in the non-carcass parts contained (in %) 8.0 Asp, 12.7 Glu, 8.8 Pro, 15.1 Gly, 8.0 Ala, 6.6 Leu, 5.6 Lys and 7.2 Arg. The protein composition was found to be rather constant for the considered live weight range.

IV.2.2 Introduction

In the general introduction of Chapter IV, three sets of data were mentioned. The first set contained the results of the 151 UCE determinations and 147 urea infusions. The second set, which is a part of the first set, were the results of the 18 UCE determinations and urea infusions on the homogenised bulls prior to slaughter. The third set consists of the compositional data of these 18 slaughtered bulls. In Chapter IV.1, the second and third dataset were combined in regression analyses to evaluate both estimation techniques (UCE and urea infusion) and to withhold the most accurate one. The results indicated that prediction of relative body composition was more accurate than absolute body composition. Moreover, both *in vivo* estimation techniques could not considerably improve the estimation of relative body composition from fLW. Therefore, the most direct and at the same time most precise technique to gather data on the changes of the body composition over a certain weight range is using the equations of the lower half of Table IV.1.10. As such, dataset 1 will not be used, since it was only intended to be used if US or UCE would have been significantly more accurate in estimating body composition than fLW.

IV.2.3 Material and methods

The compositional data of the 17 BB dm bulls originate from the trials evaluating the *in vivo* estimation techniques: UCE and urea infusion. Experimental

design, slaughtering procedure and chemical analyses have been described in detail by De Campeneere *et al.* (1999b or 2000c; Chapter IV.1).

Apart from the 17 bulls, 3 newborn BB dm calves (2 female and 1 male, between 45 and 61 kg LW), originating from the suckler cow herd of the Department, were also homogenised. These calves died just before or during the first hours after birth. They were homogenised without separating CC from NCP. Further analysis was comparable with that of the bulls.

All statistical calculations were performed using SPSS 8.0 (1998). Caloric values were calculated using regression analysis with energy content (MJ) as dependent variable and kg protein and kg fat as independent variables.

IV.2.4 Results and discussion

Body compositional data

The homogenised bulls were fed differing rations. Therefore, the influence of the treatment on the body composition was studied. Covariance analysis excluded any influence of the treatments on the compositional data. This is in agreement with the results of the parallel feeding trial (Chapter III.2), in which exactly the same four treatments were applied on 4 groups of 26 animals. The different treatments had no influence on carcass composition (estimated from rib-cut dissection). As such, all compositional data could be pooled for analysis.

In Table IV.1.2 and Table IV.1.3, data on the weight and the composition of the NCP, the CC and the EB, as well as on LBM and on fasted LW (fLW) of the 17 homogenised bulls are listed. These results showed very low fat contents in the dm animals, especially when the large range in EBW is taken into account. The compositional results were given in IV.1.4.

Earlier results of Clinquart *et al.* (1994), Fiems *et al.* (1995c) and several others indicated that the proportion of fatty tissue in BB dm bulls is very low. Fiems *et al.* (1995c) found 11.3 % fatty tissue in the carcass of BB dm bulls and 21.7 % for BB bulls with normal conformation fed the same ration. Their LW equalled 695 and 651 kg respectively. Clinquart *et al.* (1994) found for the same parameter 11.6 % for BB dm bulls (mean LW: 524 kg) and 24.2 % for non-dm bulls (mean LW: 524 kg) of the BB breed. In the same trial, Holstein bulls were involved and their carcasses contained on average 25.2 % fatty tissue (mean LW: 530 kg). In comparison, Ledger *et al.* (1973) found fatty tissue contents in the carcasses of a mixed population of Boran and Hereford x Boran steers, with a slaughter weight between 137 and 448 kg, ranging from 6.3 to 40.0 %. Karnuah *et al.* (1996) mentioned fatty tissue proportions in the carcass varying from 13 to 37.8 % for Japanese Black x Holstein steers.

With regard to fat content, Waldo *et al.* (1990) reported a lower percentage (8.1 %) than our maximum value (9.7 %; see Table IV.1.3). However, LW was lower than 300 kg in his experiment, compared with more than 600 kg in our experiment. Protein content was always higher in dm bulls (minimum 18.9 %) than reported in the literature. Both Garrett and Hinman (1969) and Andrew *et al.* (1994) cited the highest protein content (16.8 %). Protein percentages in the EB of more than 20 % were only reported

for Limousin bulls (Robelin and Geay, 1978), but fat percentages less than 5 % were never reported.

Robelin *et al.* (1979) compared results of different experiments and calculated the lipid weight in the body at two different LBM: 250 and 400 kg. For Angus or Hereford steers, lipid weight equalled 68 and 224 kg respectively, for Black & White bulls 34 and 112 kg and for Limousin bulls 23 and 48 kg, respectively. From our results we calculated that corresponding lipid weight would be 13.2 and 22.9 kg respectively. In the same study, Robelin *et al.* (1979) found chemical fat contents of 9.1 and 13.9 % in the EB of Limousin bulls at 9 (± 300 kg) and 19 months (± 700 kg) respectively, while water decreased from 66.5 towards 61.8 % respectively. From our results (Table IV.1.10) we calculated a fat and water content of 4.5 and 71.4 % at 300 kg LW and 7.8 and 67.6 % at 700 kg LW.

Although the above comparison of our results with literature is very difficult since animals were not fed the same ration, the very low fat contents of the BB dm bulls are remarkable, especially since the animals in this study were fed intensively (65 % concentrates on DM base). EB composition was largely different from most reports in the literature. These first compositional data on BB dm bulls, obviously confirm the drastic reduction in fat content in BB dm bulls even when compared to Limousins, which are known to be late maturing animals (Robelin and Daenicke, 1980) and therefore lean beef producers. Besides the genetic predisposition for a high lean percentage, there is another reason why the fat contents of our results are far lower than in some other studies. The very high fat contents in the Angus and Hereford steers are partly due to castration. Augustini and Branscheid (1995) compared German Fleckvieh bulls and steers at different LW. Tissue fat in the carcass varied from 6.9 % at 200 kg to 14.8 % at 650 kg for bulls and for steers from 8.2 to 23.7 % respectively. In Belgium, the male BB dm animals are traditionally fattened as bulls, which are known to have a markedly higher ratio of lean-to-fat than do castrated males (Hays and Preston, 1994). Another possible reason for a lower fat content at a comparable live weight, could be a difference in maturity. Owens *et al.* (1993) indicated that in general, animals have similar fat % at similar proportion of their mature weights. Hays and Preston (1994) calculated that at 68 %, 78 % and 88 % of mature weight, the carcasses of steers will contain 22 %, 26 % and 30 % fat, respectively. However, since mature weight of the BB dm bulls is not different from that of Limousin bulls (Sambraus, 1989), this can not explain the differences found. Therefore, whereas Limousin bulls are considered to be late maturing, BB bulls are indeed more likely to be extreme late maturing bulls (De Campeneere *et al*., 1999b).

Caloric values

From the results of the energy determinations on the one hand and the protein and fat determinations on the other hand, caloric values for protein and fat were calculated using regression analyses. For the total empty body, energy content of one kg protein and one kg fat was 22.91 ± 0.9 and 38.74 ± 1.53 MJ. The corresponding values for the carcass and the non-carcass parts were 23.46 ± 1.25 and 38.57 ± 3.04 MJ/kg, and 22.84 ± 0.89 and 37.54 ± 0.85 MJ/kg respectively. These values are in accordance with values reported in literature: based on 6 references (Table IV.2.1), mean caloric value

for protein and fat were found: 23.02 and 38.94 MJ/kg respectively. Schulz *et al*. (1974) used 23.85 (5.7 kcal/g) and 39.75 MJ/kg (9.5 kcal/g) for protein and fat respectively, in their calculations (see later in Table IV.2.4). They indicated however that they were not certain about the absolute value of these two figures.

Table IV.2.1: Caloric value (MJ/kg) of protein and fat in the empty body

	Type of animals	Protein	Fat
Our results	BB dm bulls	**22.91 ± 0.90**	**38.74 ± 1.53**
Literature			
Andrew *et al.* (1994)	Holstein cows	23.30	38.49
Ferrell *et al.* (1976)	Hereford heifers	23.49	39.62
Garrett and Hinman (1969)	Hereford steers	23.18	39.27
Paladines *et al.* (1963)	Sheep	22.51	39.47
Robelin and Geay (1976)	Bulls (various breeds)	22.93	39.20
Waldo *et al.* (1990)	Holstein steers	22.71	37.61
Mean of 6 references		**23.02 ± 0.37**	**38.94 ± 0.76**

Protein composition

In Table IV.2.2, data of the protein composition of the CC, the NCP and the EB of the 17 homogenised bulls are listed. The data of the amino acid composition of the protein of the NCP and the CC, and consequently the EB, indicate that there is very little variation between animals in protein composition. Although protein composition of the CC and of the NCP were quite comparable, the proportion of each amino acid in the CC protein is always significantly different from the proportion in the NCP protein, except for phenylalanine. For five amino acids (methionine, proline, glycine, isoleucine and histidine) a difference of more than 50 % was found between their proportion in the protein of CC and NCP. Glycine content in the protein of the NCP was 95 % higher than in the CC protein. That was the highest difference found. The proline content was most variable within the protein of the total EB (CV = 13 %).

In general, amino acid composition of the protein was very constant over the considered weight range (data not shown). For the carcass, only the proportion of tyrosine in the protein was significantly ($r = -0.68$; $P < 0.01$) correlated with EBW. It should be reminded that tyrosine recovery was not 100 % and therefore figures on tyrosine concentrations are less reliable. For the protein composition of the NCP, three amino acids, glycine ($r = 0.78$, $P < 0.001$), alanine ($r = 0.57$, $P < 0.05$) and tyrosine ($r = -0.66$, $P < 0.01$) were significantly correlated with EBW. As EB protein composition was calculated from carcass and non-carcass protein composition, the amino acid proportions in the EB were always intermediate between those of the two compartments. However, the proportions in the EB always tended more towards the proportions found in the CC, as the CC is the predominant part of the EB. The proportion of glycine and phenylalanine in the EB were significantly correlated with EBW: $r = 0.61$ ($P < 0.05$) and -0.72 ($P < 0.01$) respectively.

The data on the protein composition of the 17 bulls (Table IV.2.2) have shown very little influence of the LW within the considered LW range. To enlarge that range, results were added from the analyses of three newborn BB dm calves, with a mean LW of 52 kg (De Campeneere *et al*., unpublished data). The results of those analyses are shown in Table IV.2.3. When correlation coefficients are recalculated, now with the 20 observations, the proportions of 6 amino acids are significantly ($P < 0.05$) affected by EBW: cysteine, aspartic acid, methionine, isoleucine, histidine and lysine, with only the first having a P-value < 0.01. This indicates that only small changes occur in the amino acid composition of an animal during its development and growth.

In literature, very few data are available on protein composition in ruminants. Williams (1978) analysed 8 preruminant calves after total homogenisation for the composition of their protein. Gerrits *et al*. (1998) analysed Holstein Friesian x Dutch Friesian calves at 83 and 162 kg. Their results are listed in Table IV.2.3 and are quite comparable with the results of the three BB dm calves listed in the same table. This indicates that for ruminants protein composition is probably rather constant and universal. This is in agreement with Simon (1989) who concluded that whole-body amino acid composition is comparable across species.

Influence of fLW on body composition

The equations in the upper part of the Table IV.1.10 demonstrate that an increase of the fLW with 1 kg, corresponds with an accretion of on average 632 gwater, 206 g protein, 106 g fat and 43 g ash. These data are mean values for the total live weight range of this study, presuming that the tissue accreted at 350 kg has the same composition as that accreted at 700 kg. However, from the bottom part of the same table, we can calculate the accretions of the different components for different live weight intervals of 50 kg. For each LW interval, the corresponding fLW and EBW interval was calculated. Then, the body composition at the beginning and the end of the interval can be calculated and by division of the difference between both data, mean accretion of the different components over the interval can be calculated. This was done for each LW interval of 50 kg between 325 and 725 kg. The results of the accretions are shown in Table IV.2.4. Protein accretion increases from 197 g to 211 g per kg LW increase and fat increases from 73 g to 130 g per kg for the same range. The ash content of the accreted tissue increased with increasing LW from 38 to 46 g/kg growth. Gross energy content also increased, mainly due to the increase in fat accretion. The total accretion, as the sum of the four components, never equals 1000 as the components are part of the empty body, while the growth is expressed in kg LW.

Table IV.2.2: Means, standard deviations (SD) and ranges for the protein composition (%) of the carcass, the non-carcass parts and the empty body of 17 BB dm bulls

	Cys	Asp	Met	Thr	Ser	Glu	Pro	Gly	Ala	Val	Iso	Leu	Tyr†	Phe	His	Lys	Arg
Right carcass half																	
Mean	**1.08**[a]	**8.84**[a]	**2.33**[a]	**4.08**[a]	**3.71**[a]	**15.57**[a]	**5.25**[a]	**7.71**[a]	**6.61**[a]	**4.79**[a]	**4.23**[a]	**7.53**[a]	**4.08**[a]	**3.84**[a]	**3.55**[a]	**7.37**[a]	**6.45**[a]
SD	0.13	0.29	0.13	0.23	0.40	0.72	0.57	0.39	0.23	0.19	0.14	0.25	0.16	0.12	0.15	0.44	0.22
Min	0.94	8.41	2.14	3.36	2.22	14.15	4.44	7.10	6.20	4.52	3.99	7.08	3.83	3.64	3.35	6.08	5.96
Max	1.33	9.36	2.70	4.33	4.01	16.81	6.75	8.31	6.96	5.08	4.46	7.89	4.45	4.00	3.83	8.03	6.89
Non-carcass parts																	
Mean	**1.23**[b]	**8.01**[b]	**1.34**[b]	**3.45**[b]	**4.42**[b]	**12.68**[b]	**8.83**[b]	**15.06**[b]	**8.03**[b]	**4.55**[b]	**2.57**[b]	**6.63**[b]	**2.78**[b]	**3.78**[a]	**2.26**[b]	**5.59**[b]	**7.22**[b]
SD	0.16	0.44	0.11	0.22	0.24	0.74	2.12	1.43	0.38	0.32	0.19	0.45	0.29	0.21	0.16	0.31	0.36
Min	0.83	7.32	1.16	3.11	4.06	11.29	2.80	13.35	7.43	4.04	2.21	5.83	2.18	3.48	2.00	5.08	6.55
Max	1.47	8.72	1.51	3.93	4.79	13.79	12.48	18.75	8.89	5.23	2.80	7.49	3.35	4.26	2.63	6.18	7.87
Empty body																	
Mean	**1.12**	**8.63**	**2.08**	**3.92**	**3.89**	**14.83**	**6.16**	**9.59**	**6.97**	**4.72**	**3.81**	**7.30**	**3.75**	**3.82**	**3.22**	**6.92**	**6.64**
SD	0.11	0.30	0.12	0.21	0.32	0.69	0.81	0.58	0.21	0.18	0.15	0.27	0.19	0.13	0.13	0.38	0.22
Min	0.95	8.11	1.88	3.29	2.77	13.37	4.57	8.81	6.54	4.44	3.56	6.90	3.50	3.63	3.04	5.81	6.16
Max	1.33	9.06	2.38	4.21	4.19	16.00	8.26	10.70	7.31	5.05	4.04	7.79	4.18	4.05	3.47	7.41	7.15

[a,b]: means in the same column with equal superscripts are not significantly different ($P < 0.05$)

† Tyrosine recovery was only 65 %; the values corrected to 100 % are listed.

Table IV.2.3: Means, standard deviations (SD) and ranges for the protein composition (%) of the body of 3 new born BB dm calves and some data from literature

	Cys	Asp	Met	Thr	Ser	Glu	Pro	Gly	Ala	Val	Iso	Leu	Tyr	Phe	His	Lys	Arg
Belgian Blue double-muscled calves; n = 3 (52 kg)																	
Mean	**1.44**	**7.91**	**1.75**	**3.77**	**4.22**	**13.59**	**6.79**	**10.23**	**6.92**	**4.52**	**3.25**	**6.99**	**3.41**[†]	**3.73**	**2.80**	**5.99**	**6.26**
SD	0.07	0.26	0.21	0.12	0.10	0.45	0.50	0.96	0.27	0.10	0.07	0.14	0.11	0.12	0.03	0.07	0.11
Min	1.39	7.64	1.57	3.66	4.10	13.07	6.23	9.15	6.61	4.46	3.16	6.83	3.28	3.63	2.77	5.91	6.14
Max	1.52	8.14	1.98	3.90	4.29	13.90	7.19	10.95	7.11	4.64	3.30	7.10	3.48	3.86	2.83	6.06	6.33
Gerrits *et al.* (1998) (Holstein Friesian x Dutch Friesian calves: 84 kg; n = 8)																	
Mean	**1.3**	**8.7**	**2.1**	**4.1**	**4.5**	**13.9**	**7.2**	**9.9**	**7.0**	**5.0**	**3.8**	**7.2**	**3.1**	**4.0**	**3.1**	**7.1**	**7.4**
Gerrits *et al.* (1998) (Holstein Friesian x Dutch Friesian calves: 162 kg; n = 10)																	
Mean	**1.2**	**8.6**	**1.9**	**4.1**	**4.4**	**13.9**	**7.3**	**10.2**	**7.1**	**4.9**	**3.8**	**7.1**	**3.0**	**3.9**	**3.2**	**7.1**	**7.5**
Williams (1978) (preruminant Friesian calves; n = 8)																	
Mean	**1.3**	**8.1**	**1.7**	**4.0**	**4.4**	**12.9**	**8.1**	**11.3**	**7.1**	**3.9**	**2.8**	**6.9**	**2.5**	**3.6**	**2.5**	**6.4**	**7.0**

[†] Tyrosine recovery was only 65 %; the values were corrected to 100 %

In the same table results of Limousin bulls (Robelin *et al.*, 1979) and German Friesian bulls (Schulz *et al*., 1974) are listed. These results indicate that the BB dm bulls accrete on average some 3 to 4 % more protein per kg gain than the Limousin bulls. On the other hand an important increase in fat accretion was found in Limousins in comparison with the BB dm bulls. The Friesian bulls (Schulz *et al.*, 1974) accrete less protein per kg growth, especially at higher LW. For a comparable weight range, fat accretion in BB dm bulls is extremely low in comparison with the results of the Friesian bulls. The difference between the breeds increases with increasing LW. At 500 kg, the calculated fat content in one kg growth was 98 g fat for the BB dm bulls, 181 g for the Limousins and 504 g for the German Friesians.

Table IV.2.4: Accretion in g/kg growth or MJ/kg growth of the different body components of the BB dm bulls for different LW intervals in comparison with other data and % of the accreted energy as protein and fat

LW interval	Water	Protein	Fat	Ash	Energy	% of energy accretion	
	(g/kg)	(g/kg)	(g/kg)	(g/kg)	(MJ/kg)	as protein	as fat
BB dm bulls							
325 – 375 kg	654	197	73	38	7.57	64.8	35.2
375 – 425 kg	645	199	82	39	7.87	62.4	37.6
425 – 475 kg	636	201	90	40	8.17	60.4	39.6
475 – 525 kg	627	203	98	41	8.47	58.6	41.4
525 – 575 kg	618	205	106	43	8.77	56.9	43.1
575 – 625 kg	609	207	114	44	9.07	55.4	44.6
625 – 675 kg	600	209	122	45	9.38	53.9	46.1
675 – 725 kg	590	211	130	46	9.68	52.6	47.4
Robelin *et al.* (1979) (Limousin bulls)							
304 – 440 kg	632	191	104	-	8.49	52.0	48.0
440 – 543 kg	563	205	181	-	11.63	39.7	60.3
543 – 646 kg	437	194	244	-	14.31	31.8	68.2
Calculated from Schulz *et al.* (1974) (German Friesian bulls)							
152 – 267 kg	551	160	73	35	6.72	56.8	43.2
267 – 370 kg	480	173	193	43	11.80	35.0	65.0
370 – 480 kg	488	158	281	43	14.94	25.2	74.8
480 – 576 kg	318	120	504	25	22.90	12.5	87.5

When comparing both meat types (Limousin and Belgian Blue), the lower fat content and the somewhat lower protein content within each kg growth implicate a smaller net energy requirement for tissue accretion in BB dm bulls than in Limousin bulls. This is shown in Table IV.2.4 in which the energy accreted for each kg growth is listed for the different breeds and weight intervals. For BB dm bulls this energy retention varies from 7.57 to 9.68 MJ per kg growth, while for Limousins it varies from

8.49 towards 14.31 MJ. In the same table percentage of the total energy accretion, accreted as protein or fat are given for the different breeds and intervals. From these figures it is very obvious that throughout the whole LW range more than 50 % of the accreted energy is accreted as protein in BB dm bulls. For Limousins this figure decreased towards 31.8 % and for German Friesians towards 12.5 % at higher live weights.

Owens *et al.* (1995) indicated that energetically, the efficiency of fat accretion is approximately 1.7 times that of protein. But because more water is stored with deposited protein than with deposited fat, lean tissue gain is four times as efficient as accretion of fatty tissue. This means that BB bulls do not only produce more meat at the same slaughter weight, but the energy cost to accrete each kg weight during growth is also much lower. This is in agreement with Greenhalgh (1986) who concluded that the most effective way of producing lean meat without excessive fat is to use intact males of late maturing breeds, and to slaughter them while still immature. The BB dm bulls are therefore quite appropriate and efficient for beef production.

IV.2.5 Conclusion

The composition of the BB dm bulls has revealed to be quite stable and to change only slightly and linearly with increasing live weight. From the compositional data it is obvious that the BB breed has a quite unique low-fat content. This is not only interesting because the energy-input for accretion to reach a comparable slaughter weight is much smaller, but also because a larger part of the energy retained in the animal can be consumed as lean. From the results of the homogenisations, data on the composition of the accreted tissue for the different LW intervals were calculated to be used later on for determining energy and protein standards. The protein and fat accretion per kg growth varied from 197 to 211 g and from 73 to 130 g respectively between 350 and 700 kg. Energy accretion varied from 7.57 to 9.68 MJ for the same weight range.

IV.2.6 Additional calculations: N-excretion

The results of the feeding trials as described in Chapter III, included data on crude protein intake. The compositional data of this chapter indicated that the body composition (and thus N-accretion) of BB dm bulls is good predictable from their LW. As such, N-excretion can be calculated for the different treatments (n = 10) of both feeding trials, from a comparison between the N-intake and the N-accretion. It should be stressed that predicting the N-accretion from fLW results in identical N-accretions for the different treatments per kg fLW gain. As fLW was only known at the end of the trial, fLW at the beginning of the three periods was estimated as follows. At the start of the trial, fasting weight loss was supposed to be 2 % for all the groups. Fasting weight loss at the start of the second an third period was calculated by interpolation between

the initial fasting weight loss (2 %) and the fasting weight loss at slaughter (Table III.1.6 and III.2.7).

The results of the calculations of the N-excretion for the two feeding trials are given in Table IV.2.5 en IV.2.6. Daily N-excretion varied from 124 to 214 g. When the excretion is expressed in g per kg LW gain, the values vary from 95 to 152 g N. This large variation confirms that a good feeding strategy can strongly reduce N-excretion. N-excretion per kg meat accretion is 15 % lower for the HE-groups of the first trial compared to the LE-groups (209 g *vs.* 241 g). This proves that feeding high energy levels decreases N-excretion, especially if high protein levels are fed.

The lowest N-excretion is found for the groups with the LP level, but their performances were less good. However, even when N-excretion was expressed per kg gain or per kg meat accretion, the HELP group excreted least.

Table IV.2.5: N-excretion calculated for the first feeding trial (Chapter III.1)

Energy level	LE			HE		
Protein Level	LP	MP	HP	LP	MP	HP
***Total N-intake** (kg)*						
Day 0 - 84	14,0	17,8	19,8	13,2	15,4	18,0
Day 85 - 168	16,7	21,1	23,2	15,2	18,1	20,2
Day 169 - end	12,0	14,8	16,2	10,9	13,1	14,7
Total period	42,7	53,7	59,2	39,3	46,6	52,9
***Total N-accretion** (kg)*						
Day 0 - 84	3,9	4,4	4,4	3,7	4,2	4,6
Day 85 - 168	3,9	4,0	3,9	4,0	3,9	4,0
Day 169 - end	2,1	2,2	2,0	2,2	2,2	2,2
Total period	10,0	10,6	10,3	9,9	10,4	10,8
Total N-excretion (kg)						
Day 0 - 84	10,1	13,4	15,3	9,5	11,2	13,4
Day 85 - 168	12,8	17,1	19,3	11,2	14,1	16,2
Day 169 - end	9,9	12,7	14,2	8,7	10,8	12,5
Total period	32,8	43,2	48,9	29,4	36,2	42,1
Daily N-excretion (g)	143	189	214	129	159	185
Annual N-excretion (kg)	52,2	69,1	78,2	47,0	57,9	67,4
g N-excretion/kg LW gain	105	130	152	95	112	125
g N-excretion/kg meat accretion	198	243	282	181	211	235

The figures in Table IV.2.6 clearly indicate that N-excretion can indeed be decreased by adjusting the protein and energy to the needs of the animals. The both groups being fed a decreasing protein level excrete on average 200 g N per kg meat produced or 53 kg N per year, which is clearly less than the two groups of the first trial being fed a high protein level during the total period: 258 g and 73 kg respectively. From data of INRA (1978) and Geay *et al.* (1987) for Charolais bulls between 300 and 620 kg, gaining 1.25 kg daily and being fed according to the recommendations (CP

content of the DM: 13.6 %), a daily N-excretion of 146 g could be calculated being 117 g N per kg LW gain. These figures are quite comparable with our figures of the DP and DPIE groups of the second feeding trial (Table IV.2.6). However, Minet *et al*. (1996) found from N balance studies, higher N-retentions for BB dm bulls (33 % of the N-intake) than for BB nc and Holstein bulls (27 and 23 % respectively). In that study the three types were fed the same ration which might have caused an excess of protein for the Holstein and the BB nc bulls. The Holsteins excreted indeed 30 g more N per day in their urine.

From these results can be suggested that if the BB dm bull is fed according to its energy and protein needs, the N-excretion will probably be comparable with most other bulls fed according to their own energy and protein needs.

Table IV.2.6: N-excretion calculated for the second feeding trial (Chapter III.2)

	Treatments			
	NC	DP	IE	DPIE
***Total N-intake** (kg)*				
Day 0 - 84	11,1	12,6	13,2	13,2
Day 85 - 168	13,8	15,1	16,6	16,0
Day 169 - end	17,0	16,9	21,1	15,2
Total period	42,0	44,6	50,9	44,5
***Total N-accretion** (kg)*				
Day 0 - 84	3,4	3,4	3,3	3,3
Day 85 - 168	3,4	3,3	3,5	3,5
Day 169 - end	3,6	3,5	3,5	3,4
Total period	10,4	10,2	10,3	10,3
***Total N-excretion** (kg)*				
Day 0 - 84	7,8	9,1	9,9	9,9
Day 85 - 168	10,4	11,8	13,2	12,5
Day 169 - end	13,5	13,5	17,6	11,8
Total period	31,6	34,4	40,6	34,2
Daily N-excretion (g)	124	147	162	145
Annual N-excretion (kg)	45,1	53,5	59,3	52,9
g N-excretion/kg LW gain	99	108	127	107
g N-excretion/kg meat accretion	186	197	229	202

V Energy and protein standards for Belgian Blue double-muscled bulls

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V.1 General introduction

In the first part of this study, the feeding trials (Chapter III.1 and III.2), the influences of different energy and protein treatments were evaluated on the performances of the BB dm bulls. An important influence was found of the protein content on the growth rate, while the energy level influenced the feed efficiency. Very high protein needs were found during the first part of the fattening period, but our results also indicated that protein intake can decrease considerably during the rest of the fattening. Increasing the energy level did not improve growth rate, but feed conversion and carcass quality were optimised. Very few differences were found between the different treatments on the carcass and meat quality.

In the second part (Chapter IV.1) the best *in vivo* estimation technique was determined to estimate body composition. Somewhat surprising, fLW was found to be the most useful technique to predict relative body composition. Equations were determined to predict empty body chemical composition from fLW. From these equations, the accretions of the different body components were deduced at different live weights (Chapter IV.2).

In this final chapter, combining the results of the feeding trials and the compositional data should result in the determination of energy and protein standards (Figure V.2.1).

V.2 Determination of the standards

V.2.1 Abstract

Energy and protein standards were derived for Belgian Blue double-muscled bulls based on the results of two feeding trials and a trial involving serial slaughtering. The results of the serial slaughtering experiment allowed us to calculate chemical composition and energy content of the empty body, as well as protein and energy accretion at various live weights between 350 and 650 kg. Efficiency of protein deposition was derived from the feeding experiments based on marginal protein levels. It decreased from 0.53 to 0.44 with increasing live weight. Protein standards were calculated based on the protein requirements for maintenance and for protein deposition and its efficiency. The protein standards for double-muscled bulls were higher than those reported in the literature for late maturing beef breeds. Standards for energy were also derived from the same database. Net energy for maintenance was estimated, from the results of these animals that received an optimal protein level, by subtracting the net energy retained from the net energy intake. Energy standards were then determined by summation of the energy requirements for maintenance and the energy requirements for accretion at different live weights and different growth rates. They also were higher than those reported in the literature for late maturing beef breeds, partly because the energy for maintenance was surprisingly high.

V.2.2 Introduction

Robelin and Daenicke (1980) divided beef cattle into four categories: very early-maturing animals (Angus, Hereford), early-maturing animals (Friesian type), late-maturing animals (Charolais, Limousin) and dual-purpose breed animals (intermediate between early- and late maturing; Salers, Simmental). They concluded that energy and protein requirements might be different among breeds because of a different deposition of protein and adipose tissue. Double-muscled (dm) animals have a different body and carcass composition than animals with a normal conformation, especially within the Belgian Blue (BB) breed, where the double-muscling phenomenon is extremely pronounced (Boccard and Dumont, 1974; Ansay and Hanset, 1979; Fiems *et al.*, 1995b). Therefore, BB dm bulls can not be considered as one of the four types of beef cattle mentioned by Robelin and Daenicke (1980). Ménissier (1982) stated that dm animals have specific nutritional requirements that are probably different from those of normal animals. Boucqué *et al.* (1984) and Fiems *et al.* (1995a) have shown the need for extra protein in the diet of BB dm finishing bulls in comparison with animals with a normal conformation (Levy *et al*., 1980; Anderson *et al.*, 1988). Fiems *et al.* (1990) and Dufrasne *et al.* (1995) emphasised the need for a high energy content in the diet for dm bulls. However, no protein or energy standards are available up to now for these extreme late-maturing animals. This study aims to determine specific energy and protein standards for BB dm bulls during the finishing period (350 - 650 kg).

V.2.3 Material and methods

The dataset

During five consecutive years a total of 333 BB dm bulls, were involved in two feeding trials, in order to determine the influence of different energy and protein levels on the performances. Each ration consisted of concentrates and maize silage (65/35 on DM basis) and all animals were confined in straw-bedded loose houses. Overall mean live weight (LW) equalled 372 kg at the beginning and 688 kg at the end of the trials. Fiems *et al.* (1998) and De Campeneere *et al.* (1999a) described both experiments in detail (see Chapters III.1 and III.2). In both experiments, the animals of each treatment (in total: 10 treatments) were divided over different pens, with in each pen at least 3 and at most 8 animals.

Apart from these feeding trials, the chemical composition and energy content of 17 BB dm bulls were determined at different LW's ranging from 309 to 723 kg. Treatment, slaughtering procedure and the chemical analyses have been described in detail by De Campeneere *et al.* (1999c and 2000b) (see also Chapter IV.1). The results indicated that the evolution of the empty body (EB) composition, EB weight gain and the amount of protein and energy accreted with each kg increase of body weight could be estimated quite accurately based on the fLW.

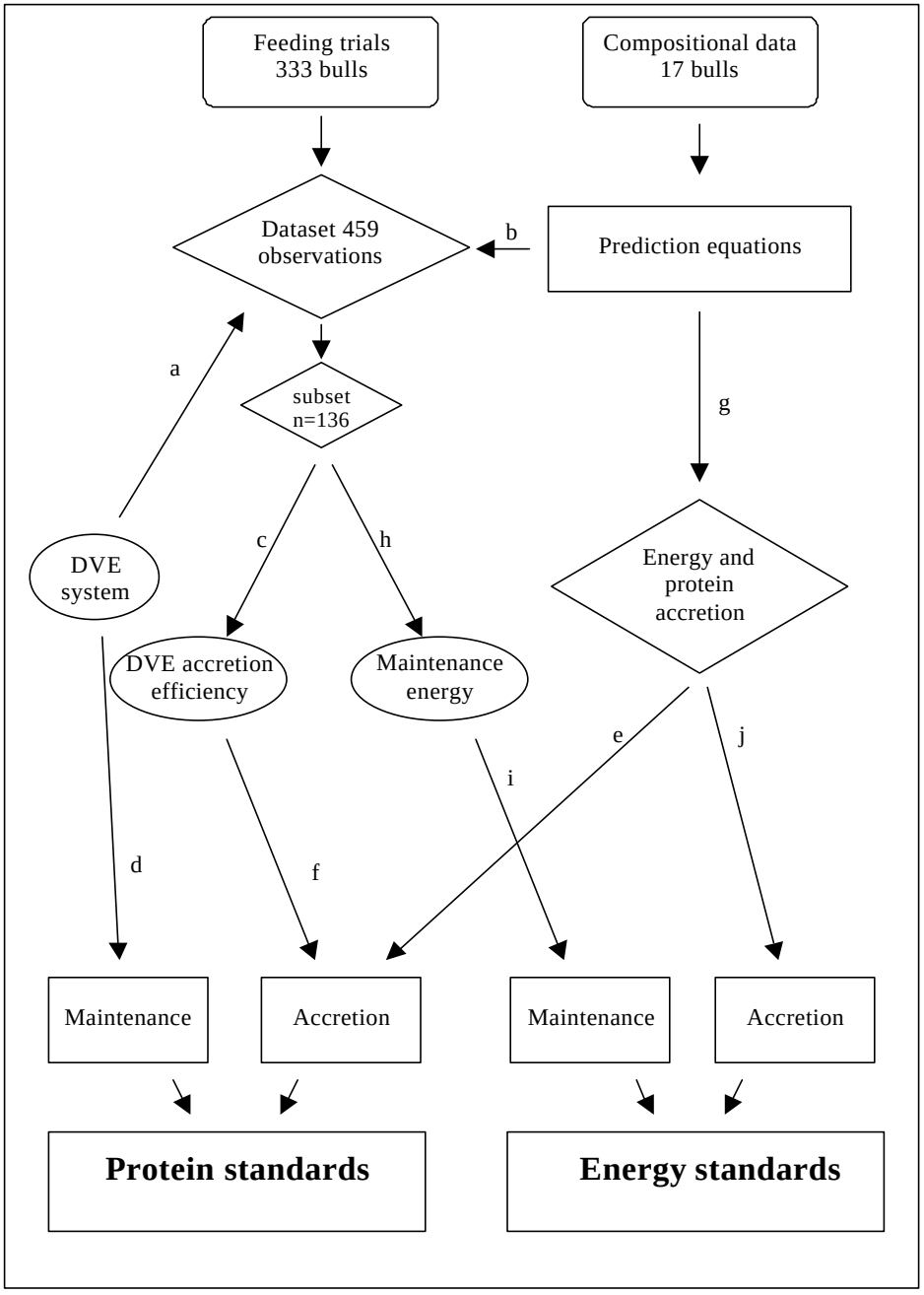

Figure V.2.I: The determination of the energy and protein standards: an overview

To determine energy and protein standards, the results of the feeding trials were calculated for each pen per interval of at least two weeks (a total of 459 observations). For each observation, LW at the beginning and at the end, daily live weight gain, daily intake of net energy for fattening (NEF; van Es, 1978) and daily DVEc-intake was known. From the LW, mean body composition of the animals of each pen at the beginning and at the end of each observation, could be estimated using the prediction equations. Based on the body composition at the start and the end of each observation, accretion of the different components (water, protein, fat, ash and energy) could be calculated. As such, protein and energy intake and accretion was known for each observation.

In Figure V.2.1 a schematic overview illustrates the determination of the protein and energy standards. The prediction equations (Table IV.1.10), derived from the compositional data, are used twice. Once, to determine the accretion of the 459 observations in the dataset originating from the feeding trials (Figure V.2.1: arrow b), and once, to determine the amount of protein and energy accreted for each kg growth (Figure V.2.1: arrow g; see Table IV.2.4).

Derivation of the protein standards

Protein standards, expressed in DVEc, were obtained using the following model: summation of the requirement for maintenance (DVEc-m) and the requirement for accretion (DVEc-a). The second term being the amount of protein accretion divided by the efficiency of protein accretion (k_{pa}).

$$\text{DVEc-standard (g/day)} = \text{DVEc-m} + \text{DVEc-a}$$

or

$$\text{DVEc-standard} = \text{DVEc-m} + \text{protein accretion}/k_{pa} \qquad (1)$$

For each observation of the dataset, maintenance requirement and protein accretion could be calculated. Protein requirements for maintenance (DVEc-m; g/day) were calculated using the formulae of NRC (1984) for endogenous urinary and scurf protein loss, as assumed in the DVE/OEB-system (Tamminga *et al.*, 1994), and using the mean LW of the observation (Figure V.2.1 arrow a):

$$\text{DVE-m} = [(2.75 \times LW^{0.5} + 0.2 \times LW^{0.6})/0.67]. \qquad (2)$$

Daily protein accretions for all the observations (mean of all the animals of that pen, varying from 3 to 8) were calculated based on their LW and the prediction equations previously reported in Chapter IV.2 (Figure V.2.1 arrow b). Subsequently, k_{pa} was derived only for these observations of the dataset, with animals fed a protein level, above which a higher DVEc content did not result in a significant increase of the growth rate (adequate minimum protein level; Geay *et al.*, 1987). For these observations (n = 136) with no shortage nor a surplus of protein, DVEc-intake approaches the DVEc standard. So, the efficiency of protein deposition in growing animals could be estimated

based on equation (1), from the daily protein intake, the daily protein accretion and the maintenance requirements (Figure V.2 arrow c). This efficiency was determined for each of the 136 observations and a mean value for each weight interval of 50 kg was then calculated.

Protein standards were finally calculated according to formula (1) with DVEc-m estimated from formula (2) (Figure V.2.1 arrow d), protein accretion estimated from the homogenisations taking the LW and the daily gain into account (Figure V.2.1 arrow e) and k_{pa} also in function of the LW (Figure V.2.1 arrow f). For each weight interval of 50 kg, between 350 and 650 kg, and for different growth rates within each LW interval, protein standards were determined and expressed as daily intake of g DVEc.

Derivation of the energy standards

The energy standards were calculated in a similar factorial way. This involves a partition of the total NEF requirements into requirements for maintenance and growth (Rohr, 1978):

$$\text{NEF-standard (MJ/day)} = \text{NEF-m} + \text{NEF-a} \quad (3)$$

with NEF-m = net energy for maintenance and NEF-a = net energy for accretion.

The energy intake was known for each observation, and the energy accretion could be calculated from the results of the homogenisations (Chapter IV.2). Energy in the empty body was estimated from the fLW using the following equation (Figure V.2.1 arrow g):

$$\text{MJ/kg EBW} = 5.754 + 0.0032 * \text{fLW}$$

$$\text{adj}R^2 = 0.36 \qquad \text{RSD} = 0.54 \text{ MJ}$$

From this equation a mean energy accretion per kg empty body weight (EBW) was calculated for each weight interval of 50 kg. Notwithstanding that the R^2-value is rather low, the RSD is acceptably low, meaning that the estimation is quite precise.

Energy for maintenance (NEF-m) was estimated based on the same 136 observations with animals fed the optimal protein level. For each of these observations, energy intake (NEF-i) approaches NEF-standard and therefore NEF-m could be calculated from the difference between NEF-i and NEF-a using the model (Figure V.2.: arrow h):

$$\text{NEF-i} = \text{NEF-m} + \text{NEF-a}.$$

A regression equation was then calculated to predict NEF-m from LW, in order to estimate the NEF-m for each LW interval of 50 kg.

Energy standards were finally calculated according to formula (3) with NEF-m estimated from the dataset (Figure V.2.1: arrow i) and NEF-a estimated from the compositional data (Figure V.2.1: arrow j). For each weight interval of 50 kg, between

350 and 650 kg, and for different growth rates within each LW interval, energy standards were determined and expressed as daily intake of MJ NEF.

V.2.4 Results and discussion

Results of the trials used for the dataset

Fiems *et al.* (1998) and De Campeneere *et al.* (1999a) described the results of the feeding trials (see Chapter III.1 and III.2). Both reports confirmed that dm finishing bulls have higher protein requirements than non-dm ones. These reports also indicated that performance and carcass quality could be optimised by decreasing the protein content in the diet with increasing LW, and by providing a high energy level in the final stage of the finishing period. For more details on the results of both trials, see Chapter III.1 and III.2.

The homogenisations indicated that body composition of BB dm bulls only varies slightly and linearly within the considered LW range and that body composition can be estimated quite accurately based on fLW. Means, standard deviations (SD) and ranges for the chemical composition and energy content of the 17 homogenised bulls are given in Table IV.1.3. For more details on the *in vivo* estimation techniques and the compositional data see Chapter IV.1 and Chapter IV.2 respectively.

The protein standards

Daily protein requirements for maintenance were calculated according to equation (2) and varied from 86.8 g DVEc to 119.2 g DVEc at 350 and 650 kg respectively. This is much lower than if calculated from the ARC-system (AFRC, 1992; 2.3 g/$LW^{0.75}$) or from the French system (Geay *et al*., 1987; 3.25 g/$LW^{0.75}$). But both systems consider the endogenous protein losses in digestion (DVMFE) as a part of the maintenance requirements, while in the DVE/OEB-system these losses are considered as a part of the feeding value.

Apart from the protein requirements for maintenance, the protein accretion and its efficiency were to be calculated. The figures for protein accretion were derived in Chapter IV.2, and vary from 197 g protein per kg growth at 350 kg to 209 g protein at 650 kg (Table IV.2.4). These data have been discussed and compared to other data in Chapter IV.2.

Finally the efficiency of protein accretion (k_{pa}) was calculated, only with those groups (n = 136) that were fed the optimal protein level, based on the protein for maintenance, the protein accretion and the protein intake. The efficiency decreased with increasing LW, from 0.53 at 350 kg to 0.44 at 650 kg (Table V.2.1).

Geay *et al.* (1987) showed that the efficiency of protein accretion was different among breeds (Table V.2.1), with a higher efficiency in the Charolais than in the Friesian breed, and that the efficiency decreased with increasing body weight. van Vliet *et al.* (1994) also used different efficiencies for different types of cattle: early, intermediate and late maturing bulls (Table V.2.1).

Table V.2.1: Efficiency of digestible protein in the intestine used for protein deposition

Live weight (kg)	100	150	200	250	300	350	400	450	500	550	600	650	700
This study													
Belgian Blue dm						0.53	0.51	0.49	0.48	0.47	0.45	0.44	
van Vliet *et al*. (1994)													
Early maturing	0.68	0.67	0.65	0.63	0.59	0.55	0.51	0.45	0.39	0.32	0.23		
Intermediate	0.69	0.68	0.67	0.64	0.62	0.59	0.55	0.50	0.45	0.39	0.31	0.22	
Late maturing	0.70	0.69	0.68	0.67	0.65	0.62	0.60	0.57	0.54	0.50	0.46	0.41	0.36
Geay *et al.* (1987)													
Friesian		0.68					0.53				0.28		
Charolais							0.64				0.48		

The Cornell Net Carbohydrate and Protein System (Fox *et al.;* 1992) uses coefficients for efficiency of 0.75 for LW's smaller than 181 kg, 0.50 from 181 to 363 kg and 0.41 above 363 kg. In comparison with Geay *et al.* (1987) and Fox *et al.* (1992), the efficiency derived from our experiment was less variable, with a relatively lower value at a lower LW and a higher value at a higher LW. According to AFRC (1992) the efficiency equals 0.59 and is independent of the LW. It is not clear why the efficiency at the lower LW's was lower in our experiment compared to other authors.

In general, a better efficiency was expected, based on the fact that dm animals are characterised by a higher percentage of white myofibres (Batjoens *et al.*, 1991), which are well-known to have a lower protein turnover than red ones (Garlick *et al.*, 1989) and based on the smaller proportion of the intestines in dm bulls. Therefore, the estimation of the protein requirements for maintenance according to the DVE/OEB system might not be totally correct for the BB dm bulls. But on the other hand, if DVE-m would be lower than according to NRC (1984), k_{pa} would even be lower.

The protein standards for different LW's and LW-gains could be calculated from the protein requirement for maintenance, the protein deposition and the efficiency of protein deposition (Table V.2.2). As our standards were based on a limited dataset, standards were only derived for these combinations of LW and daily gain which were present in the dataset. In Table V.2.3 the protein standards are expressed in DVEc per kg DM, calculated for an average DM intake for each live weight interval of 50 kg. It should be stressed that de DVEc standards have been corrected for a negative OEB. Therefore, a negative OEB is acceptable, as long as the proposed DVEc standards are reached.

Because of a relatively low protein efficiency at a lower LW and a higher protein deposition at a higher LW, a higher protein intake is required for BB dm animals in comparison with other breeds. Protein standards (DVEc) for dm bulls are not always higher than the standards of the similar AAT/PBV system (Madsen *et al.*, 1995). However, since the AAT/PBV system considers the faecal N losses as a part of the maintenance requirements and not as a characteristic of the ration, the AAT-values should be decreased by the DVMFE. Besides, within the AAT/PBV system a negative PBV (~ OEB) is tolerated, in contrast with the standards derived in this study. When the AAT standards are corrected for both remarks, they are always lower than the standards for BB dm bulls. In Table V.2.4 a comparison is made between different standards for a low and a high growth level. The standards for BB dm bulls are much higher than the standards of CVB (van Vliet *et al.*, 1994). Especially at higher growth rates the difference can reach 30 %, which is a consequence of the lower k_{pa} found in our results and the higher protein accretion. The higher protein needs are in accordance with the results of the feeding trials (Fiems *et al.*, 1998 and De Campeneere *et al.* 1999a), Boucqué *et al.* (1984) and Fiems *et al.* (1995a) who earlier indicated that BB dm bulls have higher protein requirements than non-dm bulls. Comparison with the results of INRA (1988) is very difficult, because the difference in calculation of the PDI and DVE concerning the DVMFE. For each LW interval, mean daily DVMFE was calculated from the characteristics of the rations fed in our feeding trials and the daily DM-intake.

Table V.2.2: Net energy (MJ NEF/day) and protein standards (g DVEc/day) for Belgian Blue double-muscled bulls at different growth rates

	Daily gain (g)																	
	1000		1100		1200		1300		1400		1500		1600		1700		1800	
LW (kg)	DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF
350											645	52.9	680	53.7	720	54.5		
400									640	57.8	680	58.6	715	59.3	755	60.1	795	60.9
450							630	61.9	675	62.7	715	63.5	755	64.3	795	65.1	835	66.0
500					610	65.2	655	66.1	695	66.9	740	67.8	780	68.6	825	69.5		
550	545	67.0	590	67.9	635	68.7	680	69.6	720	70.5	765	71.4	810	72.3				
600	575	69.8	620	70.7	665	71.6	715	72.5	760	73.4								
650	595	71.9	645	72.8	690	73.7	740	74.7										

Table V.2.3: Net energy (MJ NEF/kg DM) and protein standards (g DVEc/kg DM) for Belgian Blue double-muscled bulls for an average mean daily DM intake at different growth rates

		Daily gain (g)																	
		1000		1100		1200		1300		1400		1500		1600		1700		1800	
LW (kg)	Daily DM intake	DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF	DVEc	NEF
350	6.9											93	7.7	99	7.8	104	7.9		
400	7.7									83	7.5	89	7.6	93	7.7	98	7.8	104	7.9
450	8.3							76	7.4	81	7.5	86	7.7	91	7.7	96	7.8	101	7.9
500	8.8					69	7.4	74	7.5	79	7.6	84	7.7	89	7.8	93	7.9		
550	9.2	59	7.3	64	7.4	69	7.5	74	7.6	78	7.7	83	7.8	88	7.9				
600	9.4	61	7.4	66	7.5	71	7.6	76	7.7	81	7.8								
650	9.5	63	7.6	68	7.7	73	7.7	78	7.9										

Table V.2.4: Comparison of protein standards (g DVEc/day) for Belgian Blue double-muscled bulls (BB dm) and standards of other systems for two different growth levels

	Low growth level						High growth level					
LW	Growth (kg/day)	BB dm g DVEc	CVB g DVE	INRA g PDI	INRA† g DVE	ARC g MP‡	Growth (kg/day)	BB dm g DVEc	CVB g DVE	INRA g PDI	INRA† g DVE	ARC g MP‡
350 kg	-	-	-	-	-	-	1.6	680	535	695	592	-
400 kg	1.4	640	510	690	576	550	1.6	715	555	735	621	-
450 kg	1.4	675	530	730	606	-	1.6	755	580	775	651	-
500 kg	1.2	610	560	725	593	541	1.6	780	605	820	688	-
550 kg	1.0	545	480	720	583	-	1.4	720	585	825	688	-
600 kg	1.0	575	510	775	635	535	1.4	760	615	875	735	620
650 kg	1.0	595	540	835	694	-	1.2	690	595	885	744	-

† PDI corrected for DVMFE (endogenous protein losses in digestion)
‡ MP = metabolisable protein

The corrected PDI values (INRA[†] in Table V.2.4) can be compared to the DVE values. From that comparison one can conclude that for lower LW's the standards of INRA are lower, but for higher LW and low growth level, they are higher. The standards of the ARC have twice been increased with 10 %, the first time for working with bulls and the second time to have figures for a late maturing breed. The figures then have about the same magnitude as the ones of CVB, but they should also be corrected for DVMFE. Therefore, it is obvious that the standards obtained in this study are very deviant from the ones of ARC.

The energy standards

For each observation, the accreted energy could be estimated from the LW at the beginning and the end of the observation. Subsequently, since the VEVI system is a NEn system, NEF-m could be calculated by subtracting the NEF-a from the NEF-i for these observations with animals fed the optimal protein level. This was done for each observation. NEF-m (MJ per day) was best estimated using the following equation:

$$NEF\text{-}m = -13.5 + 0.205 * LW - 0.000135 * LW^2$$

$$R^2 = 0.85 \qquad RSD = 3.60$$

To compare our estimate of NEF-m with others, it was expressed as kJ/kg $LW^{0.75}$: a mean value of 507 kJ/kg $LW^{0.75}$ was found (R^2 = 0.70; RSD = 3.97). In Figure V.2.1, the calculated NEF-m values are plotted as a function of $LW^{0.75}$. That value is only based on the 136 observations being fed the optimal protein level. When the value was calculated for all observations a comparable value of 504 kJ/kg $LW^{0.75}$ was found. Very little variation was found when NEF-m was separately calculated for each of the 10 treatments of both feeding trials; NEF-m varied between 485 and 521 kJ/kg $LW^{0.75}$.

Several factors may be at the origin of the high maintenance requirements as determined here. At first, the trials were not designed for determining the maintenance requirements and therefore the estimation should be interpreted with some precaution. For the traditional determination of maintenance requirements, animals are restrained in metabolic cages. In our study, the animals were all loose-housed in groups and were fed *ad libitum*. Therefore, costs for locomotion and eating must be higher and these requirements are part of maintenance in our study. The NEF-m is as such not only a measure for the maintenance requirements, but also for eating and ruminating and digestion and even for locomotion. Besides, Vermorel *et al.* (1994) found that the average cost of standing was 25 % higher in dm than in nc calves.

Secondly, the high feeding intensity and the high rate of gain will have influenced the NEF-m. Maintenance requirements were commonly derived from studies where animals are fed close to maintenance level or even fasted, but in our trials growth rate was very high and this might have influenced our results. Frisch and Vercoe (1977) noted that steers fed *ad libitum* had proportionally a 20 % higher fasting heat production than steers restricted to 80 % of *ad libitum*. Ferrel *et al.* (1986) even found a difference of 40 % in fasting heat production between sheep fed a high and a low plane of nutrition prior to the measurements. Recently, several authors found very high estimates of the MEn for maintenance with high productive animals. Yan *et al.* (1997), Dawson and

Steen (1998) and Kirkland and Gordon (1999) determined with confined animals, MEn for maintenance to be: 670 kJ/kg $LW^{0.75}$ with lactating cows, 614 kJ/kg $LW^{0.75}$ with beef cattle and 610 kJ/kg $LW^{0.75}$ with lactating cows respectively. Yan *et al.* (1997) concluded that part of the extreme high value obtained in their study was due to the type of diet (*ad libitum* grass silage-based diets).

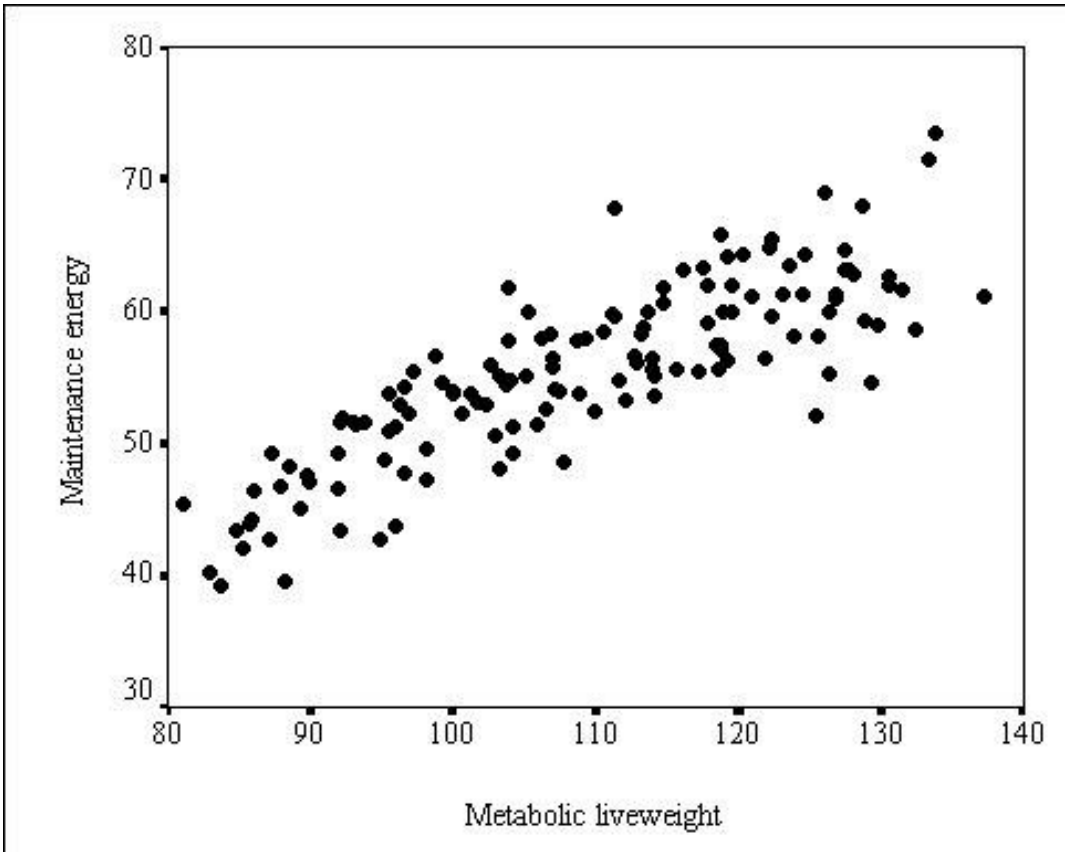

Figure V.1.1: Calculated energy for maintenance (MJ/day) in function of metabolic liveweight ($LW^{0.75}$): 507 kJ/kg $LW^{0.75}$ (R^2=0.70)

Table V.2.5: Comparison of energy standards (MJ/day) for Belgian Blue double-muscled bulls (BB dm) and of other systems for two different growth levels

	Low growth level				High growth level			
Live weight	Growth kg/day	BB dm MJ NEF	CVB MJ NEF	INRA MJ NEF †	Growth kg/day	BB dm MJ NEF	CVB MJ NEF	INRA MJ NEF†
350 kg	-	-	-	-	1.6	53.7	50.1	49.5
400 kg	1.4	57.8	50.1	49.5	1.6	59.3	54.2	54.1
450 kg	1.4	62.7	53.9	53.3	1.6	64.3	59.1	57.9
500 kg	1.2	65.2	58.0	53.3	1.6	68.6	62.5	62.4
550 kg	1.0	67.0	52.8	53.5	1.4	70.5	62.2	61.7
600 kg	1.0	69.8	56.3	56.4	1.4	73.4	66.3	66.3
650 kg	1.0	71.9	60.1	60.2	1.2	73.7	65.6	65.4

† INRA (1988)

All these values largely excel that proposed by the Agricultural and Food Research Council (AFRC, 1990) being 480 kJ MEn/kg $LW^{0.75}$. Yan *et al.* (1997) argued that the measurements of AFRC were done with steers in fasting state and prior to measurement they were offered diets at maintenance levels, while in his and the other recent studies, cattle were fed *ad libitum*. Ferrel *et al.* (1986) and Birkelo *et al.* (1991) already indicated that the plane of nutrition before determination of the requirements influences energy requirements for maintenance.

The value we found also largely excels the value of 368 kJ NEn/kg $LW^{0.75}$ (502 kJ MEn/kg $LW^{0.75}$) proposed by van Vliet *et al.* (1994) in the VEVI-system. These results are of the same magnitude as those of AFRC (1990) and originate from feeding trials described by Geay *et al.* (1987), who indicated that the value of 368 kJ should be increased with 15 % for animals with low fat contents at high live weights. This is certainly the case for the BB dm bulls. Therefore, our 507 kJ should be compared (provided comparison is possible) with 423 kJ instead of 368 kJ, the first still being much smaller than our estimation. The surplus of 15 % extra maintenance requirements is a compensation for the higher energy cost for protein turnover, since the muscle mass of these animals is much larger (van Es, 1980; Russel and Wright, 1983). Unfortunately, no further details could be found concerning the feeding and production level, the housing and other experimental conditions during determination of NEn maintenance. Therefore, no explicit reason can be given for the difference with our estimates.

Hanset *et al.* (1987) determined MEn for maintenance for BB dm bulls (between 273 and 471 kg LW) to be 548 kJ/kg $LW^{0.75}$, while for BB nc bulls (between 288 and 483 kg LW) it was 594 kJ/kg $LW^{0.75}$. Both groups were fed a concentrate ration with straw in the rack, and the growth rate of both groups was close to 1.28 kg/day. Their values also excelled the values proposed by AFRC (1990) and van Vliet *et al.* (1994). The authors stressed however that the estimations were very rough. As such straw intake from the rack was not recorded. The difference between our value and the value of Hanset *et al.* (1987) might be caused by the energy intake from the straw, the positive influence of roughage on the maintenance requirements (Yan *et al.*, 1997) and the higher growth rate in our trials.

van Es (1980) concluded that the greatest doubt on the precision of the NEF-m concerns the young rapidly growing animal, like the object of this study. It has a high rate of N-turnover and is physically rather active.

Conclusively, our results largely surpassed the figures proposed by AFRC (1990) and van Vliet *et al.* (1994). Our estimation of NEF-m overestimates the strict maintenance requirements. Furthermore, recent studies indicated that high productive animals might have strongly increased maintenance requirements. Unfortunately, a conclusive answer, whether the NEF-m is overestimated or not, cannot be given from the results of this study.

However, the fact that the animals were fed *ad libitum* and that the high energy levels positively influenced feed efficiency and some parameters of carcass quality, may indicate that dm animals need high energy intake for optimal performances. Therefore, in the calculation of the energy requirements, the high NEF-m values were maintained.

Besides, for the determination of the standards, we were not really interested in the maintenance requirements for fasted animals or animals at maintenance level (maintenance requirements in narrow sense), and although the estimation of NEF-m made in this study can probably not be compared with estimations of NEF-m at maintenance levels, it can be used for determining the standards since the standards apply to the situation in which the measurements have been made.

Energy standards, expressed in NEF (MJ/day) were calculated as the sum of NEF-m and NEF-a for the different LW intervals and different growth rates. The standards are listed in Table V.2.2. In Table V.2.3 the standards are expressed in NEF per kg DM, calculated for an average DM intake for each live weight interval of 50 kg. From this table it is clear that energy content can remain practically unchanged during the total fattening period. Since protein concentration can decrease with increasing LW, the energy/protein ratio increases.

Mainly due to the high energy requirements for maintenance, our energy standards (Table V.2.2) are higher than those reported by INRA (1988) and van Vliet *et al.* (1994) for non-double-muscled beef breeds (Table V.2.5).

V.2.5 Conclusion

Energy and protein standards were derived for Belgian Blue double-muscled bulls for live weight intervals of 50 kg between 350 and 650 kg, based on the results of two feeding trials and compositional data from an experiment involving serial slaughtering. The standards were expressed in MJ NEF and g DVEc intake per day and in MJ NEF and g DVEc per kg DM for an average DM intake.

Daily protein standards are very high at low live weights, but the protein feeding can be decreased considerably with increasing live weight. When farmers would feed their cattle according to the proposed protein standards, a significant decrease can be expected in the N-excretion of the beef cattle population, since farmers nowadays tend to feed an excess of protein.

Although the energy requirements for maintenance found in this study were surprisingly high, the standards based on these maintenance requirements do not seem to excel the daily energy intake of the animals of the feeding trials. This indicates that dm bulls have very high energy requirements for optimal performances. Therefore we believe that the energy standards proposed are justified, although the calculated energy for maintenance requirements are surprisingly high. The relatively high energy standards will have a positive influence on the feed efficiency and on some carcass characteristics, as was found in the feeding trials. Especially the positive influence of the high energy levels on the protein efficiency is of significant importance for the reduction of the N-excretion.

Concluding remarks of the study and future research

The results of two feeding trials indicated that adequate protein and energy feeding is primordial for efficient beef production. High protein levels are necessary for optimal growth rates and good carcass quality. High energy levels do not improve growth rate, but increase feed efficiency. From the two feeding trials, a general feeding scheme could be derived to optimise animal performance and carcass and meat quality.

From the trial with serial slaughtering, the first data on chemical composition of BB dm bulls were determined. As expected, the bulls are characterised by a very low fat content, even at higher live weights. The small changes in body composition that we found were very well explained by a linear regression with fLW as the independent variable.

By combining the results of the feeding trials and the compositional data, protein and energy standards were derived for fattening Belgian Blue dm bulls between 350 and 650 kg. High daily protein intakes are very important for optimal performances at lower live weights, but the protein intake can be decreased with increasing live weight. Feeding high energy levels throughout the total fattening period optimises performances and carcass quality. The combination of a reduced protein feeding with increasing live weight and improved protein efficiency, due to high energy feeding, can significantly reduce the N-excretion from the beef cattle population.

The standards derived in this study have been derived with bulls that were intensively fed from 350 kg LW on. The use of these standards is limited to BB dm bulls finished in comparable conditions.

Some questions remain unanswered and future research focusing on these problems is needed to fine-tune the standards. Especially, efforts should be made to determine the energy and protein requirements for maintenance.

Since protein requirements for maintenance could not be derived precisely from the results of this study, the formulae as proposed by the NRC (1984) were used. However, the drastic anatomic and physiologic changes suggest that also the maintenance requirements for protein might be somewhat deviant for the BB dm bull.

The energy requirements that were derived from the results of our trials were surprisingly high. The actual reason for the high maintenance requirements should be found. Do dm bulls really have very high energy maintenance requirements when fattened intensively? To answer this and other questions, separate trials should be performed to determine the protein and energy requirements for maintenance for this type of animal.

Almost as important as both previous research items, would be an independent evaluation of the energy and protein standards. Therefore, a feeding trial should be designed in which the bulls are fed according to the protein and energy standards. From the comparison of the predicted and the recorded performances, conclusions should be drawn and improvements might be suggested.

Furthermore, research concerning the influence of animal treatment during the phase preceding the fattening period might be of essential importance. To what extent can feed restriction in the phase influence the protein and energy requirements during the fattening period? How long should restricted feeding be applied to benefit maximally? A comparable problem, is the possibility to keep the animals on an extensive diet up to higher live weights (*e.g.* 500 kg), and to start fattening from that weight on. Can we still apply the same proposed standards or should they be adapted, due to compensatory gain?

From the *in vivo* estimation techniques, two important questions remain unanswered. On the one hand, the theory that urea diffuses more slowly in dm bulls due to a lower capillary density should be confirmed. On the other hand, a reason should be found why the creatinine excretion per kg body protein in dm bulls is higher than in other breeds.

Summary

Energy and protein standards for Belgian Blue double-muscled bulls were determined based on the results of two feeding trials, conducted during three and two years respectively. A total of 333 animals were involved, with a mean initial live weight of approximately 365 kg. The mean slaughter weight varied between 680 and 700 kg. Apart from the feeding trials, an experiment involving serial slaughtering was carried out to provide information on the body composition of the Belgian Blue double-muscled bulls at varying live weights.

The first trial investigated the influence of three protein levels within each of two energy levels. The results of the three series clearly indicated that protein level significantly influenced daily gain. That influence was mainly a result of an improved growth rate during the first period of the trial. Adequate protein feeding (100 g DVE or 160 g CP per kg DM) is very important during that period. Later on, when the animals weigh more than 500 kg, the protein content of the ration can be reduced considerably (77 g DVE or 125 g CP per kg DM), without negative effects on performances.

No influence of the energy content of the ration on the growth rate was found. However, the animals that received a high energy level (8.0 MJ NEF per kg DM) had an improved DM, CP and DVE conversion rate as well as an improved conformation and a reduced fasting weight loss. The energy and protein level only had very small influence on the quality of the meat.

Based on these results, the possibility to adjust protein and energy levels to the changing needs of the animal was investigated in the second trial using phase-feeding. Total experimental period was divided in three phases based on their LW: 360 - 460 kg, 460 - 570 kg and 570 - 680 kg. Unlike the negative control (constant low protein level), the three other groups received with each phase, decreasing protein (constant energy), increasing energy (constant protein) and decreasing protein in combination with increasing energy respectively. The results confirmed the main conclusion of the first trial: a high protein level (100 g DVEc or 160 g CP/kg DM) is very important until 460 kg; later on, this level can be reduced. This trial also indicated that if protein content is decreased from 460 kg onwards, it is advisable to maintain 80 g DVEc or 145 g CP in the ration until 570 kg, whereas from 570 kg onwards 70 g DVEc or 120 g CP/kg DM is sufficient. Very few differences were found between the groups receiving a decreasing protein level in combination with a continuous moderate or an increasing (low, moderate, high) energy level. Again, no important influence of the energy level on the growth rate was found. However, the high energy level again improved protein efficiency, mainly during the first period. Very few differences were found between feeding a constant moderate energy level or increasing the energy from a low to a high level, if both schemes are combined with a decreasing protein density.

Apart from the results of the feeding trials, data on the composition of the accreted tissue of BB dm bulls are indispensable to determine energy and protein standards. Therefore, two techniques to estimate *in vivo* body composition were evaluated: urea infusion and urinary creatinine excretion. Therefore, the body composition of four groups of BB dm bulls (a total of 46 bulls) was estimated at four different live weights. During the experiment 18 of those were slaughtered and homogenised rapidly after one of the four applications of both estimation techniques. Based on the results of the estimation techniques and the homogenisations, regression equations were calculated to estimate body composition. Urea infusion and creatinine excretion were found to be adequate predictors of absolute and relative body protein and body water. Estimation of body fat was less accurate. However, within the considered weight range, fasted live weight was comparably accurate in predicting empty body composition.

From the results of the homogenisations, the composition of the BB dm bulls has revealed to be quite stable and to change only slightly and linearly with increasing live weight. From the compositional data it is obvious that the BB breed has a quite unique low-fat composition. This is not only interesting because the energy-input for accretion to reach a comparable slaughter weight is much smaller, but also because a larger part of the energy retained in the animal can be consumed as lean. From the results of the homogenisations, data on the composition of the accreted tissue for the different LW intervals were calculated to be used later on for determining energy and protein standards. The protein and fat accretion per kg growth varied from 197 to 211 g and from 73 to 130 g respectively between 350 and 700 kg. Energy accretion varied from 7.57 to 9.68 MJ for the same weight range.

To determine energy and protein standards the results of the two feeding trials were calculated for each pen and per interval of at least two weeks (459 observations). For each observation the efficiency of protein accretion was calculated. Therefore, maintenance requirements were calculated according to NRC (1984) and the protein accretion was estimated from the results of the homogenisations. Based on these data and on the protein intake, the efficiency of the protein deposition could be calculated. For each weight interval of 50 kg, a mean efficiency was then derived from these groups who were fed a protein level, above which a higher DVEc content did not result in a significant increase of the growth (Geay *et al.*, 1987). The efficiency of the protein accretion for growth varied from 0.53 at 350 kg to 0.44 at 650 kg. Protein standards were finally calculated as the total of maintenance and protein accretion requirements.

The energy standards were calculated in a similar way. The energy intake was known for each case, and the energy accretion could be calculated from the regression equations found by homogenising the bulls. For the bulls being fed an optimal protein level, the difference between these two parameters is an estimation of the energy for maintenance. For each weight interval of 50 kg, a mean energy for maintenance was calculated, solely with the cases used to calculate the protein accretion efficiency. The sum of the energy for maintenance and for energy accretion is a measure for the total energy requirement. Although the net energy requirements for maintenance found in this study were surprisingly high (507 kJ/kg $LW^{0.75}$), partly due to the high feeding

intensity, that value was used to calculate the standards. Moreover, the feeding trials indicated that dm bulls have very high energy requirements for optimal performances, and that high energy feeding positively influences N-excretion.

For each weight interval of 50 kg, energy and protein standards were determined as explained above. These standards were expressed as daily intake of g DVEc and MJ NEF and as g DVEc and MJ NEF per kg DM, calculated for an average DM intake for each live weight interval of 50 kg.

The standards derived in this study not only optimise the performances of the animals but can also reduce feeding costs, by avoiding excess protein or energy feeding. The reduction of protein feeding in combination with a high energy level will be beneficial for the environment, due to the reduced N-excretion.

Samenvatting

Eiwit- en energienormen voor Belgisch Wit-blauwe dikbilstieren in de afmestfase

Op basis van twee productieproeven werden eiwit- en energienormen voor Belgisch Wit-blauwe dikbilstieren afgeleid. De twee proeven werden respectievelijk in drie en twee reeksen uitgevoerd, en er waren in totaal 333 dieren bij betrokken. De proeven startten telkens op een gewicht van ongeveer 365 kg en de dieren werden geslacht op een gemiddeld eindgewicht tussen 680 en 700 kg. Naast de productieproef werd een proef uitgevoerd die gegevens moest verstrekken over de lichaamssamenstelling van Belgisch Wit-blauwe dikbilstieren op verschillende lichaamsgewichten.

In de eerste proef werd het effect van twee energieniveaus, met binnen elk van beide drie eiwitniveaus, bestudeerd. Na drie reeksen kon éénduidig een belangrijke invloed van het eiwitgehalte op de dagelijkse groei worden vastgesteld. Het effect op de groei is volledig te wijten aan een verbeterde groei beneden de 500 kg. Tijdens deze fase is een voldoende eiwitvoorziening (100 g DVE of 160 g RE per kg DS) dan ook primordiaal. Eens de dieren zwaarder zijn dan 500 kg, kan het eiwitgehalte in het rantsoen aanzienlijk verlaagd worden (77 g DVE of 125 g RE per kg DS), zonder een negatief effect op de prestaties te veroorzaken. In diezelfde studie kon geen significante invloed van het energieniveau (1060 of 1160 VEVI/kg DS) op de groeiprestaties worden vastgesteld. Wel bleek een hoog energieniveau belangrijk om de voederomzet uitgedrukt in DS, RE en DVE en enkele belangrijke karkaseigenschappen, zoals bevleesdheid en uitvastingsverlies gunstig te beïnvloeden. Vandaar dat kan aanbevolen worden om een hoog energieniveau te voederen (1160 VEVI per kg DS). De verschillende eiwit- en energiecombinaties hadden nagenoeg geen invloed op de vleeskwaliteit.

In de tweede proef werd, inspelend op de resultaten van de eerste proef, nagegaan of aan de hand van fasevoedering beter beantwoord kan worden aan de behoeften van de dikbilstieren. De totale proefperiode was opgedeeld in drie subperiodes: 360 - 460 kg, 460 - 570 kg and 570 - 680 kg. Naast een negatieve controle (constant laag eiwitniveau), kregen de drie groepen met toenemend lichaamsgewicht respectievelijk minder eiwit, meer energie of minder eiwit in combinatie met meer energie. De resultaten bevestigden de belangrijkste conclusie van de eerste proefopzet; een hoog eiwitgehalte (100 g DVEc of 160 g CP) is zeer belangrijk tot ongeveer 460 kg nadien is het ongunstig om een hoog eiwitgehalte te blijven voederen. De resultaten van deze proef toonden wel aan dat, indien het hoogste eiwitniveau slechts aangehouden wordt tot 460 kg, in vergelijking met 500 kg in de eerste proef, het voordelig is om het eiwitgehalte nadien slechts te laten dalen tot 80 g DVEc of 145 g RE en tenslotte vanaf 570 kg verder tot 70 g DVEc of 120 g RE . Bij een dalend eiwitgehalte bleken er weinig verschillen te bestaan tussen een continu middelmatig energieniveau en een stijgend

energieniveau (van laag, over middelmatig, naar hoog). Ook in deze proef werd geen invloed van het energiegehalte op de groeisnelheid gevonden.

Naast de twee productieproeven werden ook gedurende twee jaar balansproeven uitgevoerd. Met deze proeven werd gezocht naar de meest efficiënte techniek om de lichaamssamenstelling *in vivo* te schatten. Infusie met ureum en totale urinaire creatinine-excretie waren de twee technieken die vergeleken werden. In de loop van de proef werden 18 van de 46 balansstieren gehomogeniseerd kort na toepassing van de schattingstechnieken. Uit de resultaten van de homogenisaties en van de schattingstechnieken konden regressievergelijkingen opgesteld worden ter schatting van de lichaamssamenstelling. De lichaamssamenstelling van Belgisch Wit-blauwe dikbilstieren bleek binnen het onderzochte gewichtstraject, zijnde 300 tot 700 kg, dusdanig gelijkmatig te evolueren, dat de lichaamssamenstelling het best geschat kan worden gebaseerd op het uitgevast gewicht. De twee onderzochte technieken gaven bevredigende resultaten, voornamelijk voor de schatting van de eiwit- en de vochtcomponent, terwijl de precisie van de schatting van de hoeveelheid vet duidelijk lager was.

Om eiwit- en energienormen op te stellen werden de resultaten van de beide productieproeven berekend per hok en per periode van minimaal twee weken (459 hokwaarnemingen). Voor elk van deze waarnemingen werd de efficiëntie van de eiwitaanzet berekend. Hiertoe werd enerzijds de eiwitbehoefte voor onderhoud berekend volgens NRC (1984) en anderzijds de aanzet aan eiwit geschat uitgaande van de resultaten van de homogenisaties. Rekening houdend met de eiwitopname kan dan de efficiëntie van eiwitaanzet voor elke hokwaarneming berekend worden. Per gewichtsklasse van 50 kg werd een gemiddelde efficiëntie van de eiwitaanzet berekend, op basis van die groepen die gevoederd werden op een niveau waarboven een hoger DVE-gehalte in het rantsoen niet meer resulteerde in een significante toename van de dagelijkse groei (Geay *et al.*, 1987). Dieren met een te laag eiwitgehalte hebben een verhoogde N-efficiëntie, terwijl dieren met een overdadige hoeveelheid eiwit, het teveel aan eiwit niet kunnen benutten en het overschot moeten uitscheiden ten koste van energie. De efficiëntie van de eiwitaanzet voor groei varieerde bij de te beschouwen groepen van 0,53 op 350 kg tot 0,44 op 650 kg. De totale eiwitbehoefte werd vervolgens berekend als de som van de behoefte voor onderhoud en de behoefte voor groei.

Voor de berekening van de energienormen werd een zelfde stramien gevolgd. Voor elke waarneming van de database was enerzijds de opname aan energie gekend en kon anderzijds de energieaanzet berekend worden. Voor deze tweede factor werd gesteund op de resultaten van de homogenisaties. Het verschil van beide factoren is een schatting van de energiebehoefte voor onderhoud. Per gewichtsklasse van 50 kg werd een gemiddelde onderhoudsbehoefte berekend, enkel op basis van de waarnemingen waarmee de efficiëntie van eiwitaanzet werd geschat. De totale energiebehoefte werd vervolgens berekend als de som van de behoefte voor onderhoud en de behoefte voor energieaanzet. Alhoewel de berekende energiebehoefte voor onderhoud verrassend hoog was (507 kJ/kg $LW^{0.75}$), deels omdat de stieren zeer intensief gevoederd werden, werd deze waarde toch gebruikt voor het berekenen van de energienormen. Bovendien hebben de resultaten van dit onderzoek aangetoond dat dikbillen hoge energiebehoeften hebben en blijken de

hoge energieniveaus een gunstige invloed te hebben op de N-excretie wat op zijn beurt gunstig is voor het milieu.

Voor elke gewichtsklasse van 50 kg werden aldus eiwit- en energienormen berekend voor verschillende groeisnelheden. Deze normen werden uitgedrukt in dagelijkse opname aan g DVEc en MJ (VEVI), evenals in concentraties (g DVEc en MJ (VEVI) per kg DS). De energienormen zijn ook onderaan deze pagina weergegeven uitgedrukt in kVEVI/dag en kVEVI/kg DS.

De voorgestelde normen kunnen niet alleen de prestaties van de stieren optimaliseren, maar beogen ook een reductie van de productiekosten doordat vermeden wordt dat overtollige hoeveelheden eiwit of energie worden gevoederd. De verlaagde eiwitgifte in de latere fasen van de afmestperiode en de verbeterde eiwitefficiëntie door de hoge energieverstrekking, hebben tenslotte ook een positief effect op het milieu door een verminderde N-uitstoot.

Energienormen voor BWB dikbilstieren bij verschillende groeisnelheden:
uitgedrukt in kVEVI per dag

LW	Dagelijkse groei (kg)								
(kg)	1,0	1,1	1,2	1,3	1,4	1,5	1,6	1,7	1,8
350						7,67	7,78	7,89	
400					8,37	8,48	8,60	8,71	8,82
450				8,96	9,08	9,20	9,32	9,44	9,55
500			9,45	9,57	9,69	9,82	9,94	10,06	
550	9,70	9,83	9,96	10,08	10,21	10,34	10,47		
600	10,10	10,24	10,37	10,50	10,63				
650	10,41	10,54	10,68	10,82					

uitgedrukt in kVEVI per kg DS opname

LW	kg DS	Dagelijkse groei (kg)								
(kg)	per dag	1,0	1,1	1,2	1,3	1,4	1,5	1,6	1,7	1,8
350	6,90						1,11	1,13	1,14	
400	7,68					1,09	1,10	1,12	1,13	1,15
450	8,32				1,08	1,09	1,11	1,12	1,13	1,15
500	8,83			1,07	1,08	1,10	1,11	1,13	1,14	
550	9,19	1,06	1,07	1,08	1,10	1,11	1,13	1,14		
600	9,41	1,07	1,09	1,10	1,12	1,13				
650	9,49	1,10	1,11	1,12	1,14					

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Wetenschappelijke publicaties:

De Boever, J.L., Iantcheva, N., Cottyn, B.G., De Campeneere, S., Fiems, L.O., Boucqué, Ch.V. 1998. Microbial protein synthesis in growing-finishing bulls estimated from the urinary excretion of purine derivatives. Animal Feed Science and Technology 75, 93-109.

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