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STRATEGIES FOR REDUCING NITROGEN EXCRETION FROM FARM ANIMALS: USE OF RATIONS WITH LOW PROTEIN CONTENT

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RIASSUNTO

L’aumento dei costi delle fonti proteiche e l’avvento in Europa della Direttiva Nitrati (91/676/EEC) hanno portato le tecniche di allevamento verso il contenimento dell’azoto contenuto nell’alimentazione animale, visto che l’uso di diete a ridotto tenore di proteina è considerata una delle migliori strategie per abbattere il contenuto di nitrati nelle deiezioni animali.

Questa tesi si propone di studiare l’effetto dell’uso di diete a ridotto contenuto di proteina nell’alimentazione del Vitellone Piemontese e nel suino pesante, considerandone gli effetti in termini di prestazioni di accrescimento, escrezioni azotate e qualità dei prodotti.

Il primo contributo della tesi propone l’alimentazione del Vitellone Piemontese con diete a due livelli di contenuto proteico (CP), alta proteina (HP) e bassa proteina (LP), con aggiunta o meno di 80 g/giorno di coniugati dell’acido linoleico (CLA), ovvero HP più CLA (HP_{rpCLA}) ed LP più CLA (LP_{rpCLA}). L’esperimento si è svolto su 48 vitelloni del peso vivo iniziale di 237 ± 24.4 kg, allevati in 12 box suddivisi equamente nell’uso delle 4 diete sperimentali (ogni dieta alimentava 3 box). Gli animali sono stati macellati al peso vivo di 668 ± 56.2 kg dopo 332 giorni di alimentazione. Rispetto la dieta HP, l’uso della dieta LP ha accresciuto l’efficienza azotata (azoto ritenuto/azoto assunto) da 0.17 a 0.23 (P<0.001) rispettivamente. L’efficienza azotata è stata influenzata dall’interazione CP × rpCLA (P = 0.047), con i vitelloni alimentati con le diete rpCLA che hanno dimostrato una miglior efficienza d’uso dell’azoto rispetto agli animali alimentati con le diete non contenenti coniugati dell’acido linoleico. Ciò suggerisce come l’uso di CLA aumenti l’efficienza d’uso dell’azoto e quanto possa esserne marcato l’effetto con l’uso di diete a ridotto contenuto proteico. L’importanza di tale fatto per l’alimentazione del Vitellone Piemontese è maggiormente apprezzabile laddove l’uso di nitrati per unità di superficie agricola sia contingentato dalla legislazione: l’uso della dieta LP senza o con aggiunta di CLA piò aumentare il peso vivo allevabile per ettaro dal 31 al 43% rispettivamente.

Nel secondo contributo ci si propone di testare l’abbassamento del tenore proteico e del contenuto di aminoacidi (A.A.) essenziali rispetto al convenzionale, nell’alimentazione del suino pesante, verificandone gli effetti sulle prestazione di accrescimento e sul livello di escrezione azotata. Si sono usate 4 diete isoenergetiche a livello decrescente di proteina (convenzionale: CONV; prteina medio-alta: MHP; protein medio-bassa: MLP; bassa proteina: LP) nelle quail la soia è stata sostituita da farina di cereali. Si sono eseguiti 3 cicli di ingrasso

di 80 animali ciascuno, per un totale di 240 suini allevati; gli animali sono stati allevati in 8 box ogni ciclo, suddivisi in 10 individui per box e alimentati in modo che le diete fossero ripartite equamente (la stessa dieta ogni 2 box). L'alimentazione è stata razionata second un piano alimentare di tipo industrial, passando da 2.4 kg/giorno ad inizio prova (P.V. 92 ± 10 kg) a 3.2 kg/giorno a fine prova (P.V. 167 ± 10); Lo spessore di grasso dorsale ha dato valori medi più elevati nelle diete MLP ed LP rispetto la dieta convenzionale; Il trattamento alimentare ha influenzato il tenore di escrezione azotata, che è decresciuta del 10, 20 e 24% nelle diete MHP, MLP ed LP, rispettivamente, rispetto la dieta CONV. I risultati ottenuti portano a concludere che l'uso di bassi tenori di proteina ed A.A. nell'alimentazione del suino pesante sia una strada perseguibile, senza conseguenze negative sulle prestazioni di accrescimento e sull'efficienza alimentare; tale strategia porta oltretutto ad un abbattimento dei costi alimentari e ad una diminuzione dell'escrezione azotata, aumentando di conseguenza il numero potenziale di individui allevabili per ettaro di superficie coltivata.

Il terzo contributo si propone di testare l'abbassamento del tenore proteico e del contenuto di aminoacidi (A.A.) essenziali rispetto al convenzionale, nell'alimentazione del suino pesante, verificandone gli effetti sui parametri della carcassa e sulla qualità del prosciutto. L'esperimento è la naturale prosecuzione della prova descritta nel secondo contributo prendendo in considerazione la sola fase *post-mortem*. Gli animali sono stati macellati tutti nello stesso giorno in un macello industriale, ad un età di 286 ± 1.9 giorni e la carcassa è stata sezionata nei consueti tagli commerciali. Dopo 24 ore di refrigerazione i prosciutti sino stati rifilati ed il giorno seguente inviati ad un prosciuttificio per essere avviati a stagionatura, il tutto secondo le procedure previste dal disciplinare DOP del Prosciutto di San Daniele. Per ottenere il prosciutto crudo DOP San Daniele le cosce hanno subito una fase di salagione (16 ± 1 giorni), una fase di riposo (98 ± 5 giorni) ed infine una fase di stagionatura (239 ± 24 giorni). Non si ha avuto alcun effetto significativo del trattamento alimentare sul peso dei tagli magri, mentre rispetto la dieta convenzionale, la dieta contenente meno proteina ha influenzato il dato dei tagli grassi, che in rapporto alla carcassa è passato da 0.238 a 0.244. La riduzione del contenutoproteico nella dieta ha portato ad un aumento del grasso di copertura delle cosce portandolo da 20.4 a 23.3 mm ed ha ridotto la perdita in peso percentuale durante la stagionatura del prosciutto da 0.285 a 0.275. I risultati di questo esperimento indicano che l'abbassamento del tenore di proteina nella dieta del suino pesante fino a 108 g CP/kg di mangime nella fase di finissaggio, non provoca effetti negative sulle performance di accrescimento e migliora le caratteristiche qualitative del prosciutto in stagionatura; ciò va a sommarsi ai risultati ottenuti e descritti nel secondo contributo.

Dai risultati ottenuti in questa tesi si conclude come l’uso di diete a basso tenore proteico sia una strategia valida e funzionale all’allevamento del Vitellone Piemontese e del Suino Pesante, portando benefici sotto il profilo tecnico, ambientale e nella sostenibilità economica del comparto industriale. Si osserva come per entrambe le specie prese in considerazione, l’alimentazione possa vedere la sostituzione della soia, rimpiazzandola con fonti non proteiche.

SUMMARY

Increasing cost of protein sources and in the European context the Nitrates Directive (91/676/EEC) are introducing strong pressures to reduce the nitrogen content in livestock diets, and feeding livestock with low protein diets is considered one of the major potential strategies to reduce N pollution in manure.

This thesis was aimed to study in beef cattle and in heavy pigs the effects of the use of low-protein diets on growth performance, N emission and the quality of products.

In the first contribute effects of diets differing in crude protein (CP) concentrations, top-dressed or not with 80 g/d of rumen-protected conjugated linoleic acid (rpCLA), on N efficiency of double muscled (DBM) Piemontese bulls were studied using four experimental diets being: high CP, HP; high CP plus rpCLA, HP_{rpCLA}; low CP, LP; low CP plus rpCLA, LP_{rpCLA}. Forty-eight young bulls (237 ± 24.4 kg body weight (BW)), housed in 12 pens were fed one of the four experimental diets. Bulls were slaughtered at 668 ± 56.2 kg BW, after 332 days on their diet. Compared to HP, LP increased N efficiency (N retained/N consumed) from 0.17 to 0.23 (P<0.001), likely due to more extensive N recycling. Nitrogen efficiency was also influenced by a CP × rpCLA interaction (P = 0.047), wherein bulls fed rpCLA used N more efficiently than bulls not receiving rpCLA when fed the LP diet, but had similar N efficiency when fed the HP diet. This suggests that CLA could exert some metabolic protein sparing effects, particularly under conditions of dietary CP shortage with respect to the bull requirements. The magnitude of the increase of N efficiency with LP compared to HP diets has a relevant impact on the DBM Piemontese bull production system, particularly where the load of N per unit land area is restricted by law. With respect to the conventional HP diet, use of LP without or with rpCLA would increase BW production per unit of N excreted by 31 and 43%, respectively.

The second contribution was aimed to verify if a reduction of CP and essential amino acids (AA) content of diets for finishing heavy pigs relative to conventional diets, is applicable without negative impact on growth traits and to quantify the reduction of N excretion associated to the use of a reduced dietary CP level. Four isoenergetic feed treatments (conventional: CONV; medium-high protein: MHP; medium-low protein: MLP; low protein: LP) were formulated by replacing soybean with wheat meal. A total of 240 crossbred pigs, grouped in three consecutive batches of 80 pigs each, were assigned to one of the four feed treatments on the basis of BW, sex and litter of origin (10 pigs per pen, 2 pens per treatment)

and were fed restricted amount of diets, which increased from 2.4 to 3.2 kg/d from start (92 ± 10 kg BW) to end of trial (167 ± 10 kg BW). Final backfat (BF) was thicker for pigs fed MLP and LP diets than for pigs fed CONV, due to an increased average gain in BF. Feed treatments affected the amount of excreted N, which decreased of 10, 20 and 24% for MHP, MLP and LP, respectively, relative to CONV. We conclude that a reduction of CP and AA content of diets for finishing heavy pigs is advisable, as no negative impact on growth performance and feed efficiency was observed with respect to CONV. The inclusion in feeds for heavy pigs of small or null proportion of soybean meal in early and late finishing, respectively, with a minimal supplementation of AA reduces feed costs and N excretion, thus increasing the potential number of pigs per unit of land where the maximum N load/ha is limited by law.

The third contribution, parallel to second contribution was aimed to evaluate if, relative to conventional diets, a reduction of CP and of essential amino acids (AA) content of diets for heavy pigs exert positive or negative impacts on carcass traits and ham quality. All the pigs were slaughtered in the same day at 286 ± 1.9 days old in a commercial abattoir. After 24-h of chilling, fresh hams were trimmed according to procedure the typical-shape of San Daniele ham. The day after, the trimmed hams were transferred to the processing firm were all the hams were processed according to the San Daniele ham procedure involving the following phases: salting (16 ± 1 d), resting (98 ± 5 d) and seasoning (239 ± 24 d). There were no signs of influence of the dietary treatment on the weights of the various lean cuts. With respect to CONV the treatment with the lowest dietary CP increased significantly the weight of the fat cuts and their proportion on carcass weight from 0.238 to 0.244. The reduction of the dietary CP content significantly increased ham fat covering from 20.4 to 23.3 mm and reduced total weight loss of ham from 0.285 to 0.275. The results of this experiment indicates that dietary CP contents in the early and finishing period of 117 and 108 g CP/kg respectively, do not influence negatively the growth performance and slightly improves the quality characteristics of hams. We conclude that the use of low protein diets over 90 kg BW is strongly suggested as a strategy to reduce the feeding costs, to reduce the N emission and the land surface required for manure spreading and to improve the quality characteristics of hams.

From the results presented in this thesis, it can be concluded that the use of low-protein diets represent a valid strategy, at least in DBM bulls and heavy pigs, to conjugate the needs of a technical, environmental and economic sustainability of meat industry. Both in bulls and in heavy pigs it was found that good performance and good quality of products can be achieved with an almost or total replacement of soybean meal with no protein sources.

GENERAL INTRODUCTION

Increasing cost of protein sources and in the European context the Nitrates Directive (91/676/EEC) are introducing strong pressures to reduce the nitrogen content in livestock diets (Yan et al., 2007). Before the implementation of dietary low protein diets in the livestock industry it is necessary to evaluate their possible consequences not only on growth performance, feed efficiency and N emission, but also their effects on the quanti-qualitative characteristics of animal products, and consequently on the profitability of animal farms and feed chains. Such evaluation is of particular importance in Italy, where the production of typical, or traditional, products is of great relevance.

Environmental legislation

The Nitrates Directive aims to protect water quality across Europe by preventing nitrates from agricultural sources polluting ground and surface waters and by promoting the use of good farming practices. It forms an integral part of the Water Framework Directive (2000/60/EEC) and is one of the key instruments in the protection of waters agricultural pressure.

Implementation

- Identification of water polluted, or a risk of pollution, such as surface freshwaters and groundwater, in particular those used or intended to be used as source of drinking water, containing or that will could contain (if no action is taken to reverse the trend) a concentration equal or higher than 50 mg/l of nitrates; and such as freshwater bodies, estuaries, coastal waters and marine waters, found to be eutrophic or that could become eutrophic (if no action is taken to reverse the trend).
- Designation as "Nitrate Vulnerable Zones" (NVZs) of areas of land which drain into polluted waters or waters at risk of pollution and which contribute to nitrate pollution; Member States can also choose to apply measures to the whole territory (instead of designating NVZs).
- Establishment of Codes of Good Agricultural Practice to be implemented by farmers on a voluntary basis.

  Codes should include measures limiting the periods when nitrogen fertilizers can be applied on land in order to target application to periods when crops require nitrogen and prevent nutrient losses to waters; measures limiting fertilizer application (on steeply sloping ground, frozen or snow covered ground, near waters courses, etc.) to prevent nitrate losses from leaching and run-off; requirement for a minimum storage capacity for livestock manure; and crop rotations, soil winter cover, and catch crops to prevent nitrate leaching and run-off during wet Seasons.

- Establishment of action programmes to be implemented by farmers within NVZs on a compulsory basis.

  These programmes must include measures already included in Codes of Good Agricultural Practice, which become mandatory in NVZs, and other measures, such as limitation of fertilizer application (mineral and organic), taking into account crop needs, all nitrogen inputs and soil nitrogen supply, maximum amount of livestock manure to be applied (corresponding to 170 kg nitrogen/ha/year).

- National monitoring and reporting.

  Every four years Member States are required to report on:

  - Nitrates concentration in ground-waters and surface waters;
  - Eutrophication of surface waters;
  - Assessment of the impact of action programme(s) on water quality and agricultural practices;
  - Revision f NVZs and action programme(s);
  - Estimation of future trends in water quality.

- Reports and studies

  The 4-yearly reports produced by the Member States are used as the basis for a 4-yearly report by the European Commission on the implementation of the Directive.

  In order to assist Member States in implementing the Directive and to extend scientific knowledge on best farming practices for protection of water quality and minimisation of nitrogen losses from agriculture.

Feeding low-protein diets to livestock

Feeding livestock with low protein diets is considered one of major potential strategy to reduce N pollution as manure contains nutrients that the animal has not retained in its body or products (Schiavon, 2002). A study supported by the European Commission (ERM, 2001) defined a simple methodology to help individual producers to quantify their own N emission on the basis of technical variables easy to collect at farm level (level of production, diet characteristics, number of animal reared). This encourages interventions on the characteristics of the diets to reduce nitrogen excretion. This methodology is based on the fact all the substance excreted by an animal are the result of the digestive and metabolic processes that transform the feeds into animal products and by products such as faeces and urine. The ERM (2001) model operate at animal level, but such framework was implemented into models which operate at farm level and some of these have been proposed by Schiavon et al. (2010). The use of these models permits to reduce the requirement of land for manure disposal when the farm produce an amount of N lower than that simplistically computed on the basis of the average number of animals of different categories reared in one year and standard coefficients representing the average N excretion of one head/year of that category. Standard coefficients have been defined from a survey conducted on national basis (Xiccato et al., 2005; MIPAF, 2006). However, such kind of models have a static nature, i.e. they are able to predict N excretion when both production and diet characteristics are known, and not to predict performance and the quality of the products as a function of the diet characteristics. Several energy and protein systems have been developed in the past to define nutrients requirements for various animal species and categories to achieve the best performance at animal level. However, less work have been done to define nutrient allowances for given production aims, including considerations about land requirements for manure disposal, quality of the products and, definitely, farm profitability. As land availability and the cost of protein sources are becoming limiting factors for the livestock industry the interest for studying the consequences of using low protein diets in this sector has greatly increased in the recent two decades. In addition, for some categories of animals current energy and protein systems contains numerous elements of uncertainness, both on the side of a right definition of nutrient requirements for a given level of production and on the side of feed evaluation (Schiavon and Bittante, 2012). In beef such shortcomings would be at least in part due to the fact that these systems have been developed using data from animals reared under geographic contexts, with breeds, feeds and production goals quite different from those that characterized the context of our beef meat industry (Schiavon and Bittante, 2012). A second situation of uncertainness for our meat industry regards the heavy pigs used for the dry-cured ham production the which peculiarities are quite strongly different from the pig industry of other countries.

Beef cattle

Several energy and protein systems have been published in Europe, North America and Australia for the assessment of nutritional supplements in relation to the needs of the growing cattle (Beever and Cottrill, 1994). On this basis, some opportunities to reduce N excretions were evidenced by Tamminga (2006), Todd et al., (2006), Vasconcelos et al., (2006) and

Archibeque et al. (2005). Nevertheless, the crude protein (CP) content of rations for bulls is rarely less than 12.5% (Tamminga, 2006), and in Italy CP levels around 14-15% are commonly applied with rations based on corn silage (Mazzenga et al., 2007; Xiccato, 2005; Cozzi, 2007). A reduction of the dietary CP content may adversely affect rumen microbial activity, and in turn feed intake, growth performance and the time required to reach the commercial maturity. However, it is not clear to what extent a reduction of the dietary CP of 3 to 4 percentage points with respect to conventional diets would influence the growth performance of growing bulls. It might be the case that such a strong CP reduction would exert small influence on growth performance but with a strong reduction of N excretion. This would permit to increase markedly the number of bull and meat production per unit of land in those areas where the N load is limited by law.

In this thesis we operated with double-muscled (DBM) Piemontese bulls. The reason of this choice was based on the fact that compared to other cattle, DBM subjects have less bone, less fat, more muscle, a higer dressing percentage (Ménissier, 1982; Shahin and Berg, 1985) and a reduced appetite (Trillat, 1967; Vissac, 1968; Geay et al., 1982). The low appetite and the high potential for lean growth suggested that DBM bulls require diets high in energy and protein density (Fiems et al., 1990). Thus the effects of a reduction of the dietary CP content on growth performance and N emission would be conveniently evidenced operating with this kind of bulls.

A second point of interest in this thesis regarded conjugated linoleic acid (CLA), as some farmers are using rumen protected CLA in combination with low protein diets (Schiavon et al., 2010). The use of CLA is claimed to be beneficial because it decreased fat deposition and slightly increate lean tissue growth and feed efficiency in monogastric animals (Park et al., 1997). As CLA is supposed also to exert saving effects on protein catabolism (Park et al., 1997; Pariza et al., 2001), it would be interesting to evaluate the effect of CLA in combination with low protein diets on cattle with very poor aptitude for fat gain and high aptitude for lean gain.

Pigs

Under unlimited feed energy availability and not limiting environmental conditions, body protein gain depends upon the supply of ideal protein available for growth, which should not exceed the requirement of protein for potential growth rate as defined by genetic merit and physiological maturity of the pig (Ferguson et al., 1994). Ideal protein is defined as the protein exhibiting optimal ratios between essential AA in relation to the needs of the pig

(NRC, 1998). Needs for maintenance and protein growth affect composition of ideal protein (NRC, 1998). Increases in BW affect composition of ideal protein because the needs for maintenance increase, whereas those for protein growth increase up to a maximum and decline thereafter, going to zero when the animals reach mature protein weight. The concept of ideal protein has important implications. Many studies evidenced that a decrease in the range from 30 to 40 g CP/kg feed and an adequate supply of essential amino acids did not affect growth performance and feed efficiency of pigs fed ad libitum up to 100 or 120 kg BW relative to conventional high protein feeds (Kerr et al., 1995; Kerr at al., 2003; Hinson et al., 2009). Conversely, low-protein diets un-supplemented with essential AA decreased ADG and feed efficiency with respect to control feeds or low protein feeds supplemented with essential AA, when pigs were fed ad libitum up 100 or 110 kg BW (Kerr et al., 1995; Ruusunen et al., 2007).

The special case of heavy pigs for dry-cured ham production

The pig production system in Italy is dominated by regulation for the production of Protected Designation of Origin (PDO) dry-cured ham, which imposes for pigs at slaughter at least 160 kg BW and 9 months of age. Feed restriction is required to meet average daily gain (ADG) with these targets. Protein and AA needs for finishing heavy pigs have been scarcely defined in experimental studies and commercial feeds are still formulated on the basis of empirical and practical experiences (Mordenti et al., 2003). These feeds contain on average 150 to 130 g CP/kg and 7.0 to 6.5 g lysine (lys)/kg feed from 90 to 165 kg BW (BREF, 2003). Previous investigations indicated that in this range of BW protein gain of restricted-fed heavy pigs declines from 108 to 91 g/d (Manini et al., 1997), feed intake increases and the ideal AA profile changes because of the variation of the maintenance to protein retention need ratio.

The hypothesis done in the present thesis is that in such production system concurrent decreases of dietary CP and essential AA content might exert little effects on pig growth performance in the interval 90 to 165 kg BW, carcass traits and ham quality relative to conventional diets and might lead to a strong decrease of N excretion. As a reduction of the dietary CP contents can be achieved by a partial replacement of protein feedstuffs with alternative feeds, namely cereal meals (starchy feeds), it was also considered interesting to study if such strategy would influence the growth performance of the pigs, carcass and ham quality. It was hypothesized that a the replacement of excess of protein with starch would exert positive effects by increasing the fat covering of carcass and hams, parameters which are considered to be related with weight ham losses during curing and final quality of dry-cured hams (Carnier et al., 1999; Gallo et al., 1999).

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GENERAL AIMS

This thesis was aimed to study in beef cattle and in heavy pigs the effects of the use of low-protein diets on growth performance, N emission and the quality of products. It is our opinion that implementation of feeding strategies based on the use of low-protein feed would be accepted by the operators of the meat industry only when three conditions are meet:

1) The use of low-protein diets as little influence on growth performance and feed efficiency and maintains or reduces the feeding costs;

2) The use of low-protein diets as little influence on the quality of carcass and products;

3) The use of low-protein diets strongly reduces N excretion, and within nitrates directive framework of the European Union, this would permit to reduce the need of land surface for manure disposal and/or to increase the number of animal reared per unit of surface maintaining the same N load.

Specific aims of this thesis were:

- FIRST CONTRIBUTE. To verify the hypothesis that, in DBM Piemontese bulls, N efficiency is increased by a shortage of dietary CP and by supplementation of rumen protected CLA, without impairing nutrient and energy retention. To study the effects of dietary CP and rumen protected CLA on blood metabolites and rumen ammonia N concentration, apparent digestibility of feed constituents, and energy and N balance; to evaluate possible strategies to reduce N excretion and to optimize farm production per unit cultivated land;
- SECOND CONTRIBUTE. To verify if a reduction of CP and indispensable amino acids (AA) content of diets for finishing heavy pigs relative to conventional diets, is applicable without negative impact on growth traits and to quantify the reduction of N excretion associated to the use of a reduced dietary CP level.
- THIRD CONTRIBUTE. To evaluate if, relative to conventional diets, a reduction of CP and of essential amino acids (AA) content of diets for heavy pigs exert positive or negative impacts on carcass traits and ham quality.

FIRST CONTRIBUTE

Low protein diets and rumen-protected conjugated linoleic acid increase nitrogen efficiency and reduce the environmental impact of double-muscled young Piemontese bulls

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Abstract

Effects of diets differing in crude protein (CP) concentrations, top-dressed or not with 80 g/d of rumen-protected conjugated linoleic acid (rpCLA), on N efficiency of double muscled (DBM) Piemontese bulls were studied using four experimental diets being: high CP, HP; high CP plus rpCLA, HP_{rpCLA}; low CP, LP; low CP plus rpCLA, LP_{rpCLA}. The HP diets (145 g/kg DM of CP) were similar those used commercially and the LP diets (108 g/kg DM of CP) were used to evaluate effects of lower dietary CP levels on intake, digestibility and body retention of nutrients, as well as N excretion. Forty-eight young bulls (237 ± 24.4 kg body weight (BW)), housed in 12 pens were fed one of the four experimental diets. Bulls were slaughtered at 668 ± 56.2 kg BW, after 332 days on their diet. Dry matter intake was measured daily on a pen basis and bulls were individually weighed monthly. Blood, rumen fluid and faecal samples were collected from each bull at 63, 179 and 283 days. Empty body composition at the start and the end of the feeding period was estimated using equations developed on DBM bulls and from the composition of the 5^{th} rib collected at slaughter. Nitrogen balance was computed using a mass balance approach. Lowering of the diet CP level reduced both blood urea N (P<0.001) and rumen ammonia N (P<0.001) concentrations, but did not influence organic matter digestibility or body retentions of protein or fat. Compared to HP, LP increased N efficiency (N retained/N consumed) from 0.17 to 0.23 (P<0.001), likely due to more extensive N recycling. Nitrogen efficiency was also influenced by a CP × rpCLA interaction (P = 0.047), wherein bulls fed rpCLA used N more efficiently than bulls not receiving rpCLA when fed the LP diet, but had similar N efficiency when fed the HP diet. This suggests that CLA could exert some metabolic protein sparing effects, particularly under conditions of dietary CP shortage with respect to the bull requirements. The magnitude of the increase of N efficiency with LP compared to HP diets has a relevant impact on the DBM Piemontese bull production system, particularly where the load of N per unit land area is restricted by law. With respect to the conventional HP diet, use of LP without or with rpCLA would increase BW production per unit of N excreted by 31 and 43%, respectively.

Keywords: Beef cattle, CLA, Double-muscled, Environmental impact, Nitrogen balance, Piemontese

Abbreviations*:* ADF, acid detergent fibre inclusive of residual ash; ADG, average daily gain; aNDF, neutral detergent fibre assayed with a heat stable amylase and inclusive of residual ash; AST, aspartate aminotransferase; BW, body weight; CLA, conjugated linoleic acid; CK, creatin kinase; CP, crude protein; DBM, double-muscled; DE, digestible energy; DM, dry matter; EE, ether extract; EEC, European Economic Community; ERM; Environmental Resource Management Institute; EBW, empty BW; fBW, fasted BW; GGT, gamma-glutamyl transferase; HP, high CP diet; IGF-1, insulin-like growth factor-1; lignin(sa), lignin determinate by solubilisation of cellulose with sulphuric acid; LP, low CP diet; ME, metabolizable energy; MIPAF, Ministry for agriculture and forestry policies (Italy); NDFd, aNDF digestibility; NEFA, non-esterified fatty acids; N_L, proportion of N excreted lost to air; NRC, National Research Council (USA); RE, retained energy; RDP, rumen degradable CP; rpCLA, rumen protected CLA; ST, sampling time.

Introduction

The lower feed intake and the high potential for lean growth suggest that double-muscled (DBM) bulls may require diets with increased protein density compared to conventional cattle (Arthur, 1995; Fiems et al., 1990). Boucqué et al. (1984) indicated that ration crude protein (CP) density should exceed 140 g/kg of dry matter (DM) for DBM Belgian Blue bulls. De Campeneere et al. (1999) suggested CP densities of 160, 143, and 120 g/kg of DM as adequate for DBM Belgian Blue bulls at 350 to 460, 460 to 570, and 570 to 680 kg of body weight (BW), respectively. Double-muscled Piemontese cattle had slightly lower growth rates than DBM Belgian Blue cattle, likely due to a lower mature BW (Arthur, 1995), but little is known about their dietary CP requirements. Commercially a dietary CP concentration of ~145 g/kg DM is commonly used, but constraints introduced by the Nitrates Directive of the European Economic Community (EEC, 1991), and the high cost of soybeans, are inducing farmers to use lower CP diets.

As environmental concerns force reductions in dietary CP concentrations, the role of metabolic N salvage by the animals may become critical for maintenance of acceptable levels of beef production in some areas of the world (Reynolds and Kristensen, 2008). However, a deficit of N for rumen microbes may reduce feed digestibility, feed intake and growth performance (Valkeners et al., 2008). Thus, the challenge is to increase N efficiency without impairing animal performance. The concept of nutritional synchrony presumes that the diet is the major determinant of the quantity and quality of nutrients supplied to the rumen microbial population, and so to the animal. Multiple ruminal and endogenous N pools determine nutrient availability to the rumen and animal (Hall and Huntington, 2008). Nitrogen sources which are not directly derived from dietary components can change the temporal pattern of N availability in the rumen from that predicted for the diet alone (Cole and Todd, 2008; Hall and

Huntington, 2008). A reduction of the dietary rumen degradable CP (RDP) supply can be compensated, in times, by an increase of N recycling with a reduction of urinary N losses (Reynolds and Kristensen, 2008). In situations where animal CP requirements are not well defined, such as in DBM Piemontese bulls, information can be drawn by determining the blood urea N and rumen fluid ammonia concentrations, and their variations over the growth period, as they reflect the contribution of dietary and the endogenous N sources to body N status (Satter and Roffler, 1975). It is expected that bulls which have previously experienced a sub-optimal dietary CP supply would maintain concentrations of urea in the blood, and of ammonia N in the rumen fluid, lower than those of bulls previously fed excess CP, but not necessarily lower performance. With increasing age, feed and CP intake usually increase and the same should occur for availability of endogenous N because turnover of more body protein mass and the decline of protein required for growth after the peak of lean growth. Thus, we expect that bulls fed high or low CP diets, with respect to their requirements, should have divergent blood urea N concentrations with increasing age. Such data, together with information regarding nutrient digestibility, energy and N balances can be used to evaluate the adequacy of different dietary CP levels to support animal growth.

In conventional cattle, in combination with low CP diets, some farmers add rumen protected conjugated linoleic acid (rpCLA) because CLA are thought to be beneficial by decreasing fat deposition and slightly increasing lean tissue growth and feed efficiency in non ruminants (Chin et al., 1994; Pariza et al., 2001). The effect of CLA in combination with low CP diets is of interest because CLA has been proposed to exert some protein-sparing effects (Park et al., 1997; Pariza et al., 2001). These effects might be more evident with ruminants having high protein requirements fed low CP diets. The Piemontese is an Italian beef breed (Bittante, 2011), selected mainly for high growth rate, muscularity and ease of calving, and exhibits a double-muscled trait in almost all offspring (Albera et al., 2001; Kizilkaya et al., 2003; Ribeca et al., 2009). Compared to other cattle, DBM cattle have less bone and fat, more muscle and a higher dressing proportion (Arthur, 1995; Biagini and Lazzaroni, 2011). The metabolic protein sparing effects due to rpCLA feeding might be better exploited using DBM bulls under conditions of dietary protein restriction, not only because of their great potential for lean gain, but also because they have very low fat gain potential, so that confounding effects of rpCLA on body fatness and tissue fat content are likely to be low.

Schiavon et al. (2010, 2011) reported that reducing CP from 145 g/kg DM (HP) to 108 g/kg DM (LP) did not affect final BW, average daily gain (ADG), DM intake, dressing proportion, carcass conformation or fat cover, whereas there was a CP × rpCLA interaction on feed efficiency. Rumen protected CLA improved feed efficiency when added to LP diets, but negatively affected feed efficiency when added to HP diets. The CP reduction also had no effect on the physical and chemical composition of various tissues dissected from a rib sample (Schiavon et al., 2011). Our study aimed to test the hypothesis that, in DBM Piemontese bulls, N efficiency is increased by a shortage of dietary CP and by supplementation of rpCLA, without impairing nutrient and energy retention. Effects of dietary CP and rpCLA on blood metabolites and rumen ammonia N concentration, apparent digestibility of feed constituents, and energy and N balances were determined. Strategies to reduce N excretion and optimize farm production per unit cultivated land, with reference to DBM bulls, are examined and discussed.

Materials and methods

Animals and experimental design

The reader is referred to parallel papers for major details about this experiment (Schiavon et al., 2010; 2011). Briefly, 48 intact young DBM Piemontese bulls were weighed and divided into four experimental groups of 12. The bulls were housed in 12 pens with 4 animals per pen and blocked by initial BW and age to assure homogeneity within pen and across treatments. There were 4 pens of light BW (208 ± 13.1 kg BW; 192 ± 16.7 d old), 4 pens of medium BW (240 ± 5.6 kg; 206 ± 16.7 d old) and 4 pens of heavy BW bulls (263 ± 8.6 kg; 206 ± 19.6 d old). Pens with similar initial BW were equally distributed among the four treatment groups.

After 28 d of adaptation, bulls within each group were fed *ad libitum* one of four diets formed from combination of two CP densities (HP: CP = 145 g/kg DM; LP: CP = 108 g/kg DM) and two top dressed additives, rpCLA or hydrogenated soybean oil. The two rpCLA groups received 80 g/d/head of rpCLA (SILA, Noale, Italy) and the other two groups received 65 g/d/head of hydrogenated soybean oil. The dose of CLA, close to that used by others (Gillis et al., 2004), was established to provide 6.3 and 6.1 g/d/head of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers, respectively. Rations were formulated as described by Schiavon et al. (2010). Diet ingredients were mixed and fed as a total mixed ration. The rpCLA fatty acids composition is in Schiavon et al. (2011).

Measurements, controls and analyses

All the bulls were individually weighed at 0, 120, 233 and 332 days on feed. Over the course of experiment, the amount of each feed ingredient loaded into the mixer-wagon, and the weight of the mixture uploaded into the manger of each pen were recorded daily. Orts remaining in the mangers were weighed weekly and sampled by pen. As bulls were not fed individually, DM intake and feed efficiency were computed on a pen basis. Over the course of the experiment, DM intake increased from 8.02 in the initial period to 10.21 kg DM/d in the final period, and it averaged 8.80 kg DM/d over the whole experiment, with no effects due to the treatments (Schiavon et al., 2010).

Samples of each feed ingredient of diets and orts were analyzed in triplicate for DM (# 934.01; AOAC, 2003), N (# 976.05; AOAC, 2003), ether extract (EE: # 920.29; AOAC, 2003) and ash (# 942.05, AOAC, 2003). Neutral detergent fibre (aNDF), expressed inclusive of residual ash, was determined (Mertens, 2002) with α-amylase and sodium sulphite in the ND using an Ankom^{220} Fibre Analyzer (Ankom Technology® Corporation, Macedon, NY). Acid detergent fiber (ADF), expressed inclusive of residual ash, and sulphuric acid lignin (lignin(sa)) contents were determined sequentially after aNDF determination (Robertson and Van Soest, 1981).

Actual ingredient, chemical composition and ME contents of the experimental diets were computed from the mean of the actual daily loads of feed ingredients recorded by mixer wagon computer and from the chemical analysis of each feed ingredient (Table 1). Metabolizable energy (ME) concentration was computed from actual chemical composition, according to the French energy system (Sauvant et al., 2004), and rumen degradable CP (RDP) was calculated according to the Dutch system (Tamminga et al., 1994). The ratios between RDP and fermentable organic matter in the diets were 0.154 and 0.118 for HP and LP diets, respectively. Consequently, the RDP balances, computed as described by Tamminga et al. (1994), were +2.6 and –18.6 g/kg DM for the HP and LP diets, respectively, where an RDP balance values close to zero indicates that a diet is well-balanced in terms of availability of energy and N for rumen microbes, whereas a negative value indicates a shortage of N available for microbes relative to energy.

Blood, rumen fluid and faecal samples were collected from each bull on days 63, 179 and 283 of the experiment. To evidence the contribution of N sources not directly derived from dietary components on blood urea, and consequently on rumen ammonia concentrations, the bulls were kept without feed for a period of time long enough to permit exhaustion of RDP in the rumen. Thus blood and rumen fluid samples were collected after a 12 to 15 h period without access to the diet.

Blood samples were collected from the jugular vein and stored in heparinized tubes under vacuum (Venoject, Terumo, Leuven, Belgium). Plasma was obtained by centrifugation (1500×*g*, 15 min, 4°C) and an indirect potentiometer analyser (Hitachi 911, Roche Boehringer, Mannheim, Germany) was used to estimate non-esterified fatty acids (NEFA), urea N, total protein, glucose, triacylglycerol, creatine kinase (CK), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and creatinine. A second blood aliquot, collected in vacuum tubes without anticoagulant, was immediately centrifugated (1850×*g*, 30 min, 4C°) and the supernatant was stored at -80°C and later analyzed for insulin-like growth factor (IGF-1; Immunolite one; Medical systems, Genoa, Italy).

Rumen fluid was collected and treated as described by Tagliapietra et al. (2011). Rumen fluid was filtered through three layers of cheesecloth to eliminate feed particles and was immediately analysed for ammonia N with a potentiometer (Bench pH/ion meter, Oakton Instruments, Vernon Hills, IL, USA) equipped with ammonia N specific electrodes.

Faeces, grab-sampled from each bull, were stored at -18°C and later analyzed in triplicate for aNDF, ADF and lignin(sa) (Mertens, 2002; Robertson and van Soest, 1981). Total tract apparent digestibility of OM, CP and NDF was estimated using lignin(sa) as an internal marker. True digestibility of CP was calculated by adjusting apparent digestibility of CP for metabolic faecal protein, quantified as 30 g/kg DM intake (NRC, 2001).

Body composition, nutrient retention and energy balance

All the bulls were slaughtered after 332 days of feeding at 562 ± 17.6 d of age and 668 ± 56.2 kg BW. After slaughter and 24 h *post mortem*, the 5th rib cut of each bull was collected and dissected into muscle, fat and bone. Each fraction was analyzed for moisture, CP, EE, and ash, and results are in Schiavon et al. (2011). Empty BW (EBW) was estimated as: 0.96 × 0.93 × BW (De Campeneere et al., 2001), where 0.96 is the ratio between fasted and full BW, and 0.93 is the ratio between empty and fasted BW (fBW). Empty body composition at the beginning of the experiment for each bull was predicted using equations proposed by De Campeneere et al. (2001). These equations use fBW (0.96 × BW) as the sole predictor variable and were developed using DBM Belgian Blue bulls fed rations with different ME and CP concentrations. The average protein and lipid contents of the rib collected at slaughter

(209 ± 5.1 and 77 ± 19.3 g/kg, respectively) were very close to those estimated by the equations of De Campeneere et al. (2001) at the same slaughter BW (*i.e.*, 207 ± 1.3 and 77 ± 3.1 g/kg EBW, respectively). Thus, as these equations are based only on fBW, to account for effects of CP and rpCLA on fat and protein retention at the end of the feeding period, the final body protein and lipid masses of each bull were computed assuming that EBW and rib contained the same proportions of CP and EE. Average daily nutrient retention was computed from the difference between the amounts of body protein and lipid at the end and at the beginning of feeding period. Retained energy (RE) was computed assuming 22.91 and 38.74 MJ/kg of retained protein and EE, respectively (De Campeneere et al., 2001).

The actual ME content of the rations were computed as described by Sauvant et al. (2004), using equations based on the measured digestible organic matter and other chemical components of the rations. Metabolizable energy intake was defined as ME × DM intake, and energy use efficiency was computed as the RE/ME intake.

Nitrogen balance

Nitrogen intake was computed from measured DM intake and analyzed N concentration of the diets using pen means. Apparent digested N was computed as: apparent N digestibility × N intake. Metabolic faecal N was calculated as: 30/6.25 (g) × DM intake (NRC, 2001). True digested N was computed as: apparent digested N – metabolic faecal N. Nitrogen retained was calculated as the difference between the amount of body protein/6.25, estimated at the start and end of the experiment and all data are expressed on daily basis. The following N efficiency indexes were defined: retained N/consumed N, retained N/apparently digested N, retained N/true digested N.

Implications for commercial farming

In many areas of the European Union featuring intensive livestock production, and defined as “nitrates vulnerable zones”, animal slurry disposal is subject to regulations that set a maximum threshold (170 kg/ha/yr) for the amount of N in slurry per unit agricultural land and per year (EEC, 1991). Reported values for N excretion by growing cattle vary widely, and depend on a number of factors among which breed, age, feed characteristics and dietary N concentration are major determinants (Poulsen and Kristensen, 1998; Smith and Frost, 2000). Standard coefficients of N excretion do not allow for incorporation of site-specific management practices and climatic conditions (Smith and Frost, 2000; NRC, 2003). Within the context of a European Commission funded study (ERM/AB-DLO, 1999; ERM, 2001), a methodology was developed to assist individual farmers to calculate N in slurry for various animal classes, taking into account animal type, diet and management practices and thereby to assess their compliance to the requirements of the "Nitrates Directive" (EEC, 1991). In this context it is important to draw a distinction between N excretion and N in slurry as the former refers to the total amount of N excreted (fresh), and the latter to the amount of N contained in the slurry to be spread on land after N losses in storage and transport. On this basis (ERM, 2001), N excretion can be quantified as N intake minus N retention, and N in slurry is estimated from total N excretion by discounting a proportion of N lost to the air for each animal class (N_L). Even if reservations can be raised about the use of a fixed N_L, as it changes substantially under different feeding, housing, slurry management and climatic conditions (Histrov et al., 2011), the problem remains that in practice no reliable tools to predict N_L on an individual farm basis are available (NRC, 2003; McGinn et al., 2007; Hristov et al., 2011). Public and private operators in this context need to quantify effects of feeding and management strategies on N excretion and N in slurry, to calibrate the appropriate stocking rate and to comply with constraints imposed by law. Thus, data from our experiment were used to create a quantitative answer for the DBM Piemontese bull production system. Therefore, to predict N in slurry, we used the N_L of 0.3 from the Italian regulation for beef cattle slurry disposal (MIPAF, 2006). This figure is similar to that reported by Oenema et al. (2007), who indicated that in the European Union Countries almost 0.3 of the N excreted in barns is lost during storage. As the value of N_L is influenced by the amount of N excreted in urine (Hristov et al., 2011), to allow applications of future predictive tools, faecal and urinary N excretions by a slaughter bull were also estimated as: N consumed – N digested, and N digested – N retained, respectively.

The number of bulls/ha corresponding to production of N in slurry of 170 kg/ha was computed as: 170/N in slurry produced by a bull in 332 d at an age of 562 ± 17.6 d. The BW yield equivalent to 170 kg N in slurry/ha was computed as: (final BW - initial BW) × number of bulls equivalent to 170 kg N in slurry.

Beside feeding strategies, an additional important aspect in DBM bull production system is that their body composition and carcass fatness change little with increasing BW and age (Schiavon et al., 2010). Thus, these bulls are commonly slaughtered within a wide range of BW and age (Boukha et al., 2011), although feed efficiency decreases with increasing maturity (Schiavon et al., 2010). To simulate the effect of an earlier age at slaughter of 15 months (463 ± 18.6 d, on average), compared to that adopted in the our experiment of 18.5

mo (562 ± 17.6 d, on average), consumption, retention and excretion of N by a bull were also computed using the data collected from 0 to 233 d on feed. In this case, the EBW N content at 463 d of age was estimated from 0.96 × BW measured at 233 d on feed (579 ± 51.1 kg fBW), also considering that De Campeneere et al. (2001) reported negligible deviations from linearity between EBW N mass and BW. Thus for each bull, the EBW N content at 463 d of age was estimated as: (EBW N $content_{562}$ - EBW N $content_{230}$)/(BW_{562} - BW_{230}) × (BW_{463} - BW_{230}) + EBW N $content_{230}$, where: 230, 463 and 562 are the average ages at which the BW measurements were made; the values of EBW N $content_{562}$ were the analysed CP/6.25 contents of the rib collected at slaughter (562 d); the EBW N $content_{230}$ were estimated from 0.96 × BW at the start of the experiment using the De Campeneere et al. (2001) equation. Data were averaged by pen, and the various variables of N consumption, retention and excretion, the number of bulls equivalent to 170 kg N in slurry and corresponding BW yields were computed following the computation procedure previously described.

Statistical analysis

The experimental design was completed after a prospective power analysis to establish the minimal numbers of experimental pens and number of bulls in each pen. The 12 pens, with 4 bulls/pen, were quantified, as described by Lerman (1996), as the minimal number to detect differences (P = 0.05; power = 85%) in ADG among treatments of 0.100 kg/d, with an anticipated within group SD for ADG of 0.150 kg/d.

In agreement with Robinson et al. (2006), animal was the experimental unit for blood parameters and rumen ammonia N concentration, for which time repeated measures from the same bull were available, and pen was considered the experimental unit for all pen based measurements: ME intake, energy and N balances, nutrient digestibility, retained nutrients and production indexes.

Pen based data regarding energy and N balances, and the production traits (12 pen observations, with 4 bulls/pen) were analysed using PROC GLM of SAS (2005) considering the effects of feeding treatment (3 df) as combinations of the two levels of dietary CP and the two of rpCLA, the pen block (B; 2 df) that considers animals of lighter, medium, or heavier initial BW to be fixed sources of variation, and the pen within Treatment and B as the error term (6 df).

Pen based digestibility data, with measurements repeated three times (36 observations), were analysed using a mixed effect model employing the PROC MIXED of SAS. The model included fixed effects of Treatment and B which were tested on the pen (within Treatment and B) $error_1$ (6 df), and effects of sampling time (ST; 2 df) and ST × Treatment interaction which were tested on the residual $error_2$ (16 df).

Data with repeated individual observations (*i.e.*, blood parameters and rumen ammonia N concentration; 144 observations), were analyzed using a mixed effect model which considered Treatment, B, ST and ST × Treatment interaction as fixed effects. Bull nested within the Treatment × B interaction was used as animal $error_1$ (36 df) for testing Treatment and B, whereas within bull variance was used for testing ST and ST × Treatment interaction ($error_2$; 88 df).

In each model, orthogonal contrasts were used to test, using the appropriate error term, effects of CP and rpCLA, and the interaction of CP × rpCLA.

Results

Blood profile, rumen fluid ammonia N concentration, and apparent digestibility

The urea N concentration of blood (Table 2) was much lower on the LP compared to the HP diet (-53%, P<0.001), it was affected by a ST × Treatment interaction (P<0.001; Figure 1). A low concentration of dietary CP increased NEFA (P<0.001) concentration of blood but did not influence other blood metabolites. Addition of rpCLA did not affect the concentration of any metabolite, although NEFA concentration tended (P = 0.06) to be lowest with addition of rpCLA (0.21 *versus* 0.24 meq/l; P = 0.06). Sampling time affected many blood parameters (P<0.001). Among these, in the 3 successive times respectively, total blood protein was 72.4, 75.8 and 79.1 g/l, glucose was 5.5, 4.4, and 4.8 mmol/l, creatinine was 160, 197 and 229 UI/l, and IGF-1 was 239, 341 and 348 ng/ml. For total blood protein a ST × Treatment interaction also occurred (P<0.001), but the effect was quantitatively small.

Rumen ammonia N was influenced by the ST × Treatment interaction (P<0.001; Figure 2). With LP, rumen ammonia N in the three successive sampling times oscillated between 2.2 to 3.4 mg/100 ml, whereas with HP it increased from 6.6 to 11.4 mg/100 ml.

The apparent digestibilities of organic matter and aNDF were not affected by diet or time (Table 3), but digestibility of CP was lower for the LP diet fed bulls than the HP diets (P = 0.014). However estimated true digestibility of CP did not differ. Addition of rpCLA seemed to exert a positive effect on organic matter and CP digestibility with LP but a negative effect on organic matter and CP digestibility of HP diets.

Energy balance

The digestibility based ME content calculated for the various rations (Table 4) averaged 11.2 MJ/kg DM. This value was somewhat lower than the value of 11.8 MJ/kg DM computed on the basis of the chemical composition of feed ingredients. Over the course of the experiment ME intake averaged 99 MJ/d, with no impact of dietary treatments.

Estimated body retention of protein and lipid, which averaged 219 and 105 g/d, respectively, were not affected by the treatments. The corresponding amounts of RE ranged from 8.8 to 9.8 MJ/d among diets, and the RE/ME intake ratio averaged 0.092, with no difference due to treatment.

Nitrogen balance

As expected, differences due to dietary CP concentration occurred when aspects of the N balance were examined (Table 5). Over the course of the entire feed period, N consumption was higher on the HP compared to the LP diet (203 versus 152 g/d; $P<0.001$). The amounts of N apparently digested were 138 and 91 g/d ($P<0.001$), and true digested N values were 179 and 134 g/d on the HP and LP diets, respectively. There were no difference in the amount of N retained, which averaged 35 g/d. The amounts of N excreted were about 167 and 118 g/d on the HP and LP diets, respectively ($P<0.001$). All N efficiency variables were influenced by the dietary CP concentration ($P<0.001$) and, over the course of the feeding period, N efficiency averaged 0.17 and 0.23 for bulls fed HP and LP diets ($P<0.001$). The reduction in dietary CP from 145 to 108 g/kg DM resulted in a 25% decrease in N consumption and a 30% drop in N excretion.

No rpCLA effect and CP × rpCLA interaction occurred for intakes of crude and digestible or true digestible N, but interactions occurred for the N efficiency variables. In particular, rpCLA addition interacted with dietary CP on efficiency of N retention when any of the crude ($P = 0.047$), the apparently digested ($P = 0.034$) or the true digested ($P = 0.027$) N intakes were considered, rpCLA increased N efficiency when added to the LP diet and not when added to the HP diet.

Implications for commercial farming

Considering the data of bulls finished at 18.5 mo (562 ± 17.6 d) of age (whole feeding period), the reduction of the dietary CP concentration did not reduce the faecal N excretion but it reduced urinary N excretion by 55% ($P < 0.001$) and the amount of N in slurry produced per bull by 29.6% from 39.2 to 27.6 kg (Table 6). When the reduction of the dietary CP concentration was applied to bulls finished at 15 mo (463 ± 18.6 d) of age, N excreted in urine was reduced by 54%, and N in slurry by 30.2% from 25.0 to 17.5 kg/bull. As a consequence, with bulls finished at 15.0 and 18.5 mo of age and fed LP without rpCLA, BW production/ha would increase relative to HP, by 27.8% and 31.5%, respectively and, for bulls fed LP_{rpCLA}, the BW production/ha would increase with respect to HP_{rpCLA} by 46.2% and 50.5%, respectively.

Discussion

Effects of dietary CP levels

Increasing evidence supports the view that a number of metabolic and physiological changes occur to conserve N when dietary CP concentrations are reduced (Cole and Todd, 2008). N recycling in the rumen could play an important role in regulating the amount of rumen available N, as it would permit maintenance of synchrony between N and energy yielding substrates for rumen microorganisms (Valkeners et al., 2004; Cole and Todd, 2008). However, a deficit in RDP could reduce ruminal and total tract digestibility and, consequently, lower DM intake and, in turn, overall performance. Valkeners et al. (2008) found that a reduction in the RDP balance from +5.3 to -23.7 g/kg DM resulted in a linear decrease in voluntary DM intake, rumen volatile fatty acid concentration and ADG, but did not influence ruminal and total tract digestibilities of organic matter, aNDF and starch, or efficiency of microbial protein synthesis in the rumen. In our experiment the reduction of the RDP balance, exclusively caused by a reduction of the level of soybean meal in the diet, did not influence voluntary DM intake (Schiavon et al., 2010), and there was no reduction of total tract apparent digestibility of organic matter and aNDF. Apparent digestibility of CP was reduced (-9%), but this reduction appeared to be mainly due to the contribution of metabolic faecal CP, as true digestibility of CP was unaffected by the dietary CP concentration.

Valkeners et al. (2008) found that an increase of the calculated RDP balance from -23.7 to +5.3 g/kg DM increased plasma urea N from about 1.9 to 8.0 mg/100 ml in young 300 to 443 kg BW DBM Belgian Blue bulls, and they also found that such an increase of the RDP balance increased ADG from 1.66 to 1.94 kg/d. Some studies show that maximum rumen fermentation occurs 2 to 4 h after feeding (Valkeners et al., 2008). At this time, the urea concentration in the blood and, in particularly, ammonia concentration in the rumen fluid are predominantly influenced by the dietary supply of RDP and by its degradation (Valkeners et al., 2008), and the endogenous N contribution can be masked. Valkeners et al. (2008) showed with two negative RDP balance diets of -9.2 and -23.7 g/kg DM, blood urea N variations throughout the 12 h after the meal small, and even nonexistent, whereas cattle fed diets with a positive RDP balance diet of +5.3 g/kg DM had variations of blood urea N throughout the 12 h after the meal. We hypothesized that the contribution of N not directly derived from dietary components on blood urea, and consequently on rumen ammonia concentrations, could be better shown by keeping the bulls without feed for a period of time long enough to permit exhaustion of RDP. Thus, concentrations of blood urea N and rumen fluid ammonia N in our experiment mainly reflect that part of N which is not directly from rumen degradation of feed N compounds. In such conditions it is expected that bulls which have previously experienced a sub-optimal CP supply would maintain concentrations of urea in blood, and of ammonia in rumen fluid, lower than those of bulls previously fed excess CP. In our experiment the blood urea N of bulls fed the LP diets was constant, and lower than 2 mg/ml, with increasing days on feed, whereas with bulls fed the HP diets, urea N increased from 2.0 to 5.0 mg/100 ml. Consistently, rumen ammonia N concentration of bulls fed LP diets was lower than 4 mg/100 ml with increasing days on feed, whereas with those fed HP, rumen ammonia N increased from 6.6 to about 11.4 mg/100 ml. Interestingly, Satter and Roffler (1975) indicated that when the rumen ammonia N concentration was less than 1 to 2 mg/100 ml, nearly all ammonia N was incorporated into bacterial cells, whereas when ammonia N was in excess of 5 mg/100 ml, no further effect on microbial protein production was evident. In our experiment, the divergent blood urea N and rumen ammonia N concentrations observed between diets with different CP levels with increasing days on feed reflect different mechanisms of N salvage as, in LP fed bulls, the lower blood urea N concentration was likely due to an increase of rumen uptake of urea by portal drained viscera and saliva (N recycling), or even to lower labile protein reserves which can be mobilized (Biddle et al., 1975). The low concentrations of blood urea N and rumen ammonia N during the entire feeding period, and the lack of effects on DM intake and nutrient digestibilities suggests that in bulls fed the LP diets the low supply of RDP was sufficiently compensated by an increase of recycled N or by other N conservation mechanisms. In this regard it is likely that the LP fed bulls grew less than the HP ones (1.5

versus 1.3 kg/d, respectively; *P* = 0.003) but only during the first 120 d, from 280 to 448 kg BW, and not over the whole feeding period (*P* = 0.59).

The ADG of our DBM Piemontese bulls (1.17 kg/d; Schiavon et al., 2010) was comparable to that found in a survey conducted on 804 DBM young Piemontese bulls from 109 sires finished on 124 farms in the Piemonte region of Italy (Boukha et al., 2007; 2011). This figure is somewhat lower than that commonly achieved in bulls of other beef breeds. For example, Xiccato et al. (2005) reported an ADG of around 1.40 kg/d for bulls of French breeds under similar environmental and feeding conditions. The lower growth rate of DBM Piemontese bulls compared to conventional bulls is mainly due to the very low fat accretion, even at high BW's (Arthur, 1995; Schiavon et al., 2010). In this regard, it is notable that NRC (2000) assumes that the fat proportion at slaughter is 0.280 of EBW. Using this value and assuming 0.040 ash content and a water to protein ratio of 3.24 (Amer et al., 1997), it can be estimated that the EBW protein content of conventional cattle is 0.160. De Campeneere et al. (2001) found an EBW fat content of only 0.074 in young DBM bulls, which is 4 fold lower than the NRC estimate (NRC, 2000). Assuming an EBW fat content of 0.077, that found in our experiment and, employing methodology proposed above, the EBW protein content of DBM bulls at about 668 kg BW should be approximately 0.208, or the value in our experiment. Based on these figures, and assuming 22.91 and 38.74 MJ/kg of retained protein and EE, the average RE of DBM bulls is about half than that of conventional cattle, 7.7 *versus* 14.5 MJ/kg, but the proportion represented by protein energy is more than double of that of conventional cattle (0.61 *versus* 0.25). Even if, in DBM cattle, the protein requirement/unit net energy for growth should be much higher than in conventional cattle, in our experiment no effect of dietary CP or RDP concentrations occurred on protein, lipid, water and ash retentions, or on the chemical composition of the rib from which the retentions were estimated. As a consequence, the N efficiency was 30% higher in LP compared to HP fed bulls. Such an improvement in efficiency also suggests that the HP diet provided DBM bulls CP in excess of requirements, at least when the entire growing-finishing period is considered.

Collectively, results suggest that the CP requirement was low during the last 100 d of the feeding period, and thus the 105 g CP/kg diet was adequate, whereas during the first 120 days of the early growing period such a dietary CP concentration was inadequate. During the last part of the feeding period the HP fed bulls reduced their growth rate, likely because they were approaching their plateau of mature BW, which probably gave the LP fed bulls a chance to recover body constituents compared to HP bulls. The reason why most commercial breeders prolong the fattening periods to 18 to 20 mo of age is, in many cases, due to the need to dilute the very high cost of the stocker calf on a heavier carcass, and to the very slow increase in fat covering (Schiavon et al., 2010). Thus we conclude that, for DBM Piemontese bulls, a reduction in the dietary CP concentration from 145 to 108 g/kg DM, corresponding to reductions of RDP from 90.8 to 68.0 g/kg DM and of rumen undegradable protein from 54.3 to 40.2 g/kg DM, does not impair growth performance and nutrient, including energy, body retentions, but reduced N excretion by about 30%, despite the low appetite and the high potential for lean growth of these bulls.

Effect of the CP × rpCLA interaction

One of the most interesting aspect of CLA supplementation in monogastric animals is its ability to reduce body fat while enhancing lean body mass. Among the various CLA isomers, two have been implicated as biologically active, the *cis*-9, *trans*-11 isomer seems to enhances growth and feed efficiency, whereas the *trans*-10, *cis*-12 CLA isomer has repartitioning properties that reduce body fat content in monogastric organisms (Pariza et al., 2001; Pariza, 2004). The reduction in body fat appears to be mainly caused by a reduction in body fat accretion, and not by mobilization of body fat which has already accumulated (Pariza, 2004). Park et al. (1997) and Pariza et al. (2001) also suggested that muscle mass may be preserved or enhanced as a result of CLA induced changes in regulation of some cytokines which affect skeletal muscle catabolism and immune function. In dairy cows, milk fat synthesis is progressively reduced by increasing supplemental amounts of the *trans*-10, *cis*-12 CLA isomer in the diet (Baumgard et al., 2001), and the commercial rpCLA used in our study was found to be effective in reducing milk fat content of cows (Dal Maso et al., 2008). Very little information about effects of CLA on growth and body composition is available for growing cattle (Gillis et al., 2004; Poulson et al., 2004). In our experiment, the potential of the *trans*-10, *cis*-12 CLA isomer to reduce body fatness was likely not fully exploited because fat deposition in DBM bulls was very low, and the effects of rpCLA on N efficiency were more likely due to action of the *cis*-9, *trans*-11 isomer.

Few studies have been conducted to investigate effects of rpCLA on total tract digestibility of feed constituents. In sheep, Huang et al. (2009) found that diets supplemented with 0.01 of rpCLA, as free acids or calcium salts, did not influence the volatile fatty acid profile of rumen fluid and digestibility of DM and of CP compared to control. In agreement with these results, in our experiment no differences due to rpCLA or a CP × rpCLA interaction on blood metabolite concentrations and nutrient digestibility were found. However, digestibility of organic matter and CP tended to be influenced by the CP × rpCLA interaction wherein rpCLA nominally increased digestibility in HP fed bulls and decreased digestibility on those fed LP. Nevertheless N efficiency was influenced by the CP × rpCLA interaction because bulls fed rpCLA converted N more efficiently when fed LP compared to HP. These results suggest that CLA could be involved in a metabolic regulation which increases efficiency of N retention, particularly under conditions of dietary protein shortage.

Implications for commercial farming

Our N balance results indicated that CP and age at slaughter exerted relevant effects on efficiency of N retention, and hence on N excretion. Faecal N excretion by a bull finished in 332 d, and slaughtered at 562 d, was not influenced by the CP content of the diet and it averaged 21.2 kg, whereas urinary N excretion was half with LP compared to HP fed bulls. Many studies have shown that urea and other urinary N compounds can be easily converted in volatile compounds and lost in the air, and it is expected that N losses in the air will increase with increasing urinary N excretions (Histrov et al., 2011). It is desirable that tools for predicting emission of N compounds in the air, and applicable at farm level, will be developed in the future. To achieve this goal, besides the cited effects of feeding, housing, slurry management and climate conditions, another key variable to be considered is the volume of the slurry in which the excreted fecal and urinary N compounds are diluted. Some mathematical models to predict fresh and mature volumes of slurry produced on farms have been proposed for other species (Schiavon et al., 2009) but, to our knowledge, no reliable models are available for growing cattle.

Our data were used to evaluate strategies to optimize the DBM Piemontese bull production system within the regulatory framework of the nitrates directive of the European Union (EEC, 1991). Expressing values of total N excretion (*i.e.*, feces + urine) on an annual basis and considering 14 d for empty periods of the barn [0.167 kg/d × 332 × 365/(332+14)] results in N excretion per DBM Piemontese bull finished on the HP diet in 332 d of about 58 kg N, a figure which is within the range found by others (Poulsen and Kristensen, 1998; Smith and Frost, 2000; ERM, 2001; Xiccato et al., 2005; Biagini and Lazzaroni, 2011). Under the country-specific regulatory context used here, where a legal figure of 0.3 N_L is assumed, N in slurry produced by one DBM Piemontese bull in 332 d (from 230 to 562 d of age and from 237 to 668 kg BW) is about 39.2 kg, so that 170 kg N in slurry are equivalent to 4.3 finished bulls with 1691 kg of BW yield/ha/yr. The reduction of dietary CP from 145 to 108 g/kg DM allows a strong reduction of N in slurry from 39.2 to 27.6 kg/bull produced, without any relevant effect on growth performance and carcass traits. This would permit an increase to 6.2 bulls/ha of agricultural land for slurry application in one year, with annual BW yields of 2380 kg/ha.

Our data also shows that during the last 100 d, when bull BW increased from 574 to 668 kg, ADG decreased to 0.8 kg/d and feed efficiency worsened, whereas body fatness did not change with respect to values in the prior growing periods (Schiavon et al., 2010). The reduction of the age at slaughter of bulls, from 18.5 to 15.0 mo, decreases N excretion and N in slurry and, with HP fed bulls, the magnitude of these reductions would permit an increase in the number of bull finished/ha in one year from 4.3 to 6.8, with an increase of BW yield of +395 kg/ha. With LP fed bulls this would permit an increase from 6.2 to 9.8 bulls finished/ha/yr, with a further increase of BW yield of +474 kg/ha. Therefore, combining reductions of dietary CP concentration and age at slaughter would permit an increase in the number of bulls finished/ha from 4.3 to 9.8 (+127%) in one year with a corresponding increase of BW yield from 1691 to 2855 kg (+69%). In the DBM Piemontese bull production system, the relevance of such figures should be carefully evaluated in terms of economic returns, taking into account the high costs of the young DBM stock bulls and the carcass characteristics required by the local meat markets.

Conclusions

We found that 145 g/kg DM of dietary CP of is not required by DBM Piemontese bulls, even if feed intake is somewhat lower than in conventional continental bulls, energy retention is lower, and protein retention is comparable to, or greater than, that of such bulls. Compared to conventional HP diets, feeding LP diets does not influence the digestibility of feed constituents but markedly improves N efficiency. Apart from a reduction in feed costs, because of the very low proportion of soybean meal in the LP diet, use of LP diets causes a large reduction (-30%) of N excreted without any negative impacts on growth performance and nutrient retention. In geographical areas where the maximum N load/ha is limited, use of LP diets can markedly increase N efficiency of DBM Piemontese bulls and, within the regulatory framework of the Nitrates Directive, the amount of meat produced per unit cultivated land would also increase. It was found that both low CP diets and rpCLA supplementation positively affected N efficiency, supporting the hypothesis that rpCLA exerts some protein-sparing effect, but only when dietary CP is low. From an economic aspect, use of rpCLA for a long time at the dose employed in our experiment is unlikely. Further research employing lower doses and/or shorter periods of administration is suggested.

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Table 1. Actual ingredient and chemical composition of experimental diets differing in CP concentration with or without addition of a rumen protected CLA[a].

	HP and HP_{rpCLA}	LP and LP_{rpCLA}
Total mixed ration ingredients (g/kg DM)[b]:		
Corn grain, ground	353	389
Corn silage	255	281
Sugar beet pulp, dried	108	120
Soybean, meal (480 g/kg CP solvent)	125	35
Wheat, bran	58	64
Wheat, straw	62	68
Vitamin and mineral mixture[c]	25	27
Calcium soap[d]	7	8
Soybean oil, hydrogenated	7	8
Chemical composition (mean ± SD) [e]:		
Starch (g/kg DM)	352 ± 24.7	387 ± 27.9
aNDF (g/kg DM)	291 ± 14.3	307 ± 15.8
CP (g/kg DM)	145 ± 8.2	108 ± 5.5
ME (MJ/kg DM)	11.8 ± 0.18	11.6 ± 0.21

[a] HP = rations containing 145 g CP/kg DM; LP = rations containing 108 g CP/kg DM; rpCLA = 80 g/d of top dressed with a rumen protected conjugated linoleic acids (rpCLA; SILA, Noale, Italy).

[b] Computed from actual daily loads of feed ingredients recorded by mixer wagon computer.

[c] Containing per kg: 120 g of Ca, 56 g of Na, 17 g Mg, 16 g P, 240,000 IU of vitamin A, 15,000 IU of vitamin D3.

[d] A product based on hydrogenated palm oil (Hidropalm; NOREL, Madrid, Spain).

[e] Chemical composition and ME contents were computed from the mean of the actual daily loads of feed ingredients recorded by mixer wagon computer and from the chemical analysis of each feed ingredient (*n* = 3; Sauvant et al., 2004).

Table 2. Blood metabolites of double-muscled young Piemontese bulls fed diets differing for CP concentrations, supplied or not with a commercial rumen protected CLA mixture (rpCLA).

	Treatment[a]				SEM	*P*				
	HP	HP_{rpCLA}	LP	LP_{rpCLA}		CP	rpCLA	CP×rpCLA	ST[b]	ST×Treatment[b]
Urea N (mg/l00 ml)[c]	3.8	3.9	1.8	1.8	0.11	<0.001	0.69	0.67	<0.001	<0.001
Total protein (g/l)	75	77	75	76	1.2	0.85	0.19	0.80	<0.001	<0.001
Glucose (mmol/l)	4.8	4.8	5.2	4.8	0.18	0.26	0.31	0.43	<0.001	0.84
NEFA (meq/l)	0.21	0.18	0.27	0.25	0.017	<0.001	0.06	0.71	0.10	0.20
Triacylglycerol (mmol/l)	0.19	0.17	0.16	0.16	0.013	0.08	0.34	0.54	0.028	0.003
CK (UI/l)	316	258	334	339	48.2	0.30	0.58	0.50	0.86	0.15
AST (U/l)	91	89	87	95	3.2	0.62	0.45	0.11	<0.001	0.08
GGT (UI/l)	18	18	19	17	0.9	0.96	0.62	0.36	<0.001	0.42
Creatinine (UI/l)	190	192	198	201	6.2	0.16	0.71	0.96	<0.001	0.77
IGF-1 (ng/ml)	308	286	316	326	14.5	0.11	0.69	0.28	<0.001	0.72

[a] HP = rations containing 145 g CP/kg DM; LP = rations containing 108 g CP/kg DM; rpCLA=80 g/d of top dressed rpCLA (SILA, Noale, Italy). [Each number is the mean of 12 observations repeated 3 times. Blood samples were collected after a 12 to 15 h period when the bulls were without feed.]

[b] ST = sampling time, blood was sampled from each bull at 63, 179, and 283 d of the feeding period.

[c] See figure 1 for the form of the interaction.

Table 3. Apparent digestibility of organic matter, aNDF and CP, and true digestibility of CP of double-muscled young Piemontese bulls fed diets differing for CP concentrations, supplied or not with a commercial rumen protected CLA mixture (rpCLA).

	Treatment[a]				SEM	*P*				
	HP	HP_{rpCLA}	LP	LP_{rpCLA}		CP	rpCLA	CP×rpCLA	ST[b]	ST×Treatment[b]
Organic matter	0.72	0.74	0.72	0.70	0.011	0.07	0.42	0.07	0.36	0.95
aNDF	0.54	0.53	0.51	0.52	0.017	0.16	0.53	0.93	0.16	0.49
CP	0.62	0.67	0.60	0.57	0.017	0.014	0.54	0.07	0.10	0.88
CP true digestibility[c]	0.83	0.87	0.88	0.85	0.017	0.55	0.85	0.07	0.40	0.68

[a] HP = rations containing 145 g CP/kg DM; LP = rations containing 108 g CP/kg DM; rpCLA=80 g/d of top dressed rpCLA (SILA, Noale, Italy). Each data is the mean of 3 pen observations (with 4 animal per pen) repeated 3 times. Apparent digestibility was assessed using lignin(sa) as the internal marker.

[b] ST= Sampling time, faeces were collected at 63, 179, and 283 d of the feeding period.

[c] The true digestibility of CP was computed as: (apparent digestible CP intake + metabolic faecal losses of CP)/CP intake. Metabolic faecal CP was estimated as: 30 (g) × DM intake (NRC, 2001).

Table 4. Metabolizable energy intakes, estimated body protein, lipid and energy retentions and energy efficiency of double-muscled young Piemontese bulls fed diets differing for CP concentrations, supplied or not with a commercial rumen protected CLA mixture (rpCLA).

	Treatment[a]				SEM	*P*		
	HP	HP_{rpCLA}	LP	LP_{rpCLA}		CP	rpCLA	CP×rpCLA
ME content (MJ/kg DM)[b]	11.3	11.5	11.2	10.9	0.32	0.25	0.92	0.33
ME intake (MJ/d)	99	100	100	95	3.1	0.55	0.45	0.40
Body retentions:								
Protein (g/d)	226	215	212	224	6.3	0.73	0.90	0.13
Fat (g/d)	119	99	102	99	11.2	0.47	0.35	0.47
Energy (RE, MJ/d)	9.8	8.8	8.8	9.0	0.44	0.42	0.38	0.24
RE/ME intake	0.098	0.088	0.088	0.095	0.0060	0.73	0.76	0.22

[a] HP = rations containing 145 g CP/kg DM; LP = rations containing 108 g CP/kg DM; rpCLA = 80 g/d of top dressed rpCLA (SILA, Noale; Italy). [Data are the mean of 3 pen observations with 4 bulls/pen.]

[b] Actual ME content of the rations computed from the measured digestible organic matter concentration and the chemical composition of the rations (Sauvant et al., 2004).

Table 5. Estimated N balance and N efficiency of double-muscled young Piemontese bulls fed diets differing for CP concentrations, supplied or not with a commercial rumen protected CLA mixture (rpCLA).

	Treatment[a]				SEM	*P*		
	HP	HP_{rpCLA}	LP	LP_{rpCLA}		CP	rpCLA	CP×rpCLA
N balance, g/d:								
Consumed	204	201	154	151	2.8	<0.001	0.25	0.92
Apparently digested	137	138	98	84	5.2	<0.001	0.25	0.20
Metabolic faecal N[b]	42	42	43	42	0.8	0.52	0.31	0.95
True digested	178	180	141	126	5.6	<0.001	0.23	0.23
Retained	36	34	34	36	1.0	0.73	0.90	0.13
Excreted	168	166	121	115	2.1	<0.001	0.13	0.49
Ratios:								
Retained /consumed	0.18	0.17	0.22	0.24	0.005	<0.001	0.19	0.047
Retained/digested	0.26	0.25	0.35	0.43	0.017	<0.001	0.10	0.034
Retained/true digested	0.20	0.19	0.24	0.29	0.009	<0.001	0.10	0.027

[a] HP = rations containing 145 g CP/kg DM; LP = rations containing 108 g CP/kg DM; rpCLA = 80 g/d of top dressed rpCLA (SILA, Noale; Italy). [Data are the mean of 3 pen observations with 4 bulls/pen.]

[b] Estimated as: 30/6.25 (g) × DM intake (NRC, 2001).

Table 6. Faecal and urinary N excreted and N in slurry produced by a double-muscled Piemontese young bull (kg/bull) fed diets differing for CP concentrations, supplied or not with a commercial rumen protected CLA mixture (rpCLA), and finished at 562 or 463 d of age. [The number of young bulls (*n*) and BW (kg) yields equivalent to 170 kg N in slurry.]

	Treatment[a]				SEM	*P*		
	HP	HP_{rpCLA}	LP	LP_{rpCLA}		CP	rpCLA	CP×rpCLA
Faecal N excretion by a finished bull[b]:								
562 d old	22.6	21.0	18.8	22.5	2.75	0.49	0.53	0.15
463 d old	14.6	13.7	12.3	14.4	1.71	0.47	0.56	0.17
Urinary N excretion by a finished bull[b]								
562 d old	33.8	34.6	21.5	16.1	2.87	<0.001	0.21	0.11
463 d old	21.3	22.0	13.5	9.7	1.99	<0.001	0.23	0.10
N in slurry produced by a finished bull[b]:								
562 d old	39.5	38.9	28.2	27.0	0.50	<0.001	0.14	0.50
463 d old	25.1	24.9	18.0	16.9	0.26	<0.001	0.047	0.11
Number of bulls equivalent to 170 kg N in slurry[c]:								
562 d old	4.3	4.4	6.0	6.3	0.12	<0.001	0.19	0.35
463 d old	6.8	6.8	9.4	10.1	0.16	<0.001	0.07	0.10
BW production equivalent to 170 kg N in slurry[d]:								
562 d old	1736	1647	2282	2479	59.2	<0.001	0.38	0.05
463 d old	2128	2045	2720	2990	85.7	<0.001	0.30	0.08

[a] HP = rations containing 145 g CP/kg DM; LP = rations containing 108 g CP/kg DM; rpCLA = 80 g/d of top dressed rpCLA (SILA, Noale, Italy). [Data are the mean of 3 pen observations with 4 bulls/pen.]

[b] Faecal N, urinary N and N in slurry were computed for finishing periods of 332 d (whole feeding period) or 233 d (first two growth periods). Faecal N was computed as: N intake × N digested/N intake; Urinary N was computed as: N digested – N retained; N in slurry was computed as: (N intake - N retained) × (1 - N_L), where N_L is the proportion of total N excreted lost to the atmosphere. [Data in the table were computed assuming N_L = 0.3, the value from current regulations for disposal of beef cattle slurry in Italy (MIPAF, 2006). It is expected that on HP diets, due to higher urinary N excretion, the N_L value would increase (Histrov et al., 2011).]

[c] Computed as: 170 /N in slurry produced per finished bull, considering finishing periods of 332 or 233 d. [The value of 170 kg is the maximum annual load of N in slurry permitted/ha of agricultural land in areas vulnerable to nitrates (EEC, 1991).]

[d] Computed as: (final BW– initial BW) × bulls produced equivalent to 170 kg N in slurry. Final and initial BW were measured at 332 or 233 d on trial, corresponding to 562 ± 17.6, and 463 ± 18.6 d of age).

Figure 1. Blood urea N concentrations during the experiment as influenced by dietary CP (high CP, HP, dotted lines; low CP, LP, continuous lines) top dressed (○) or not (●) with rumen protected CLA with measurements repeated at 63, 179 and 283 days of feeding. [Data points are the mean of 12 observations; error bars indicate SEM.] See Table 2 for statistical implications.

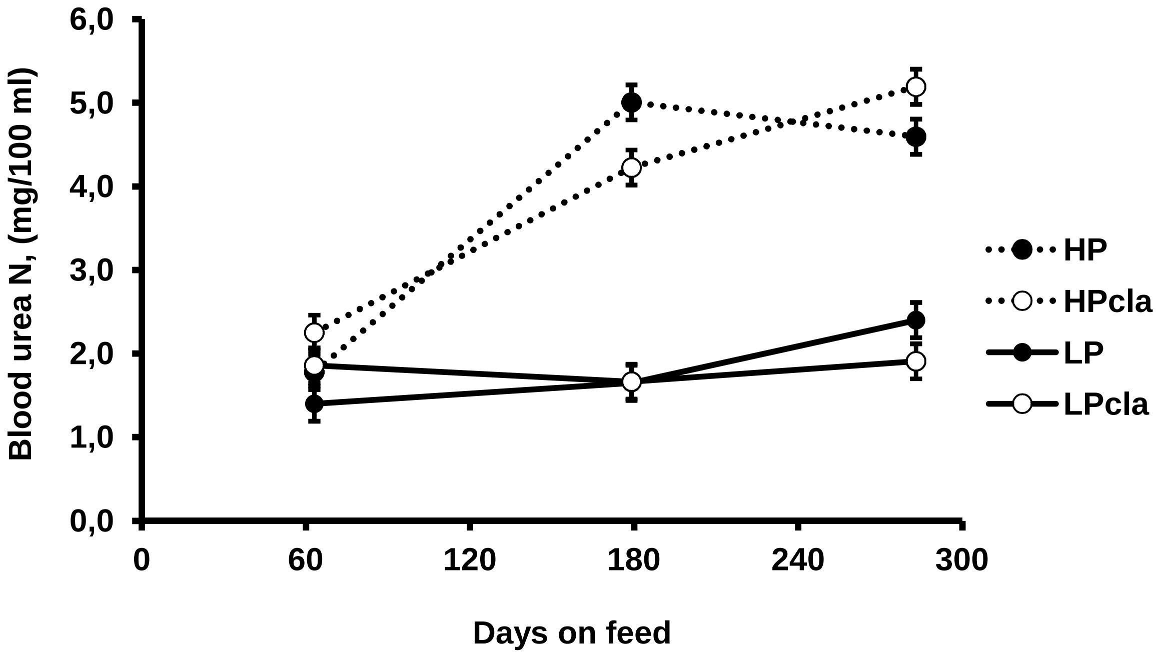
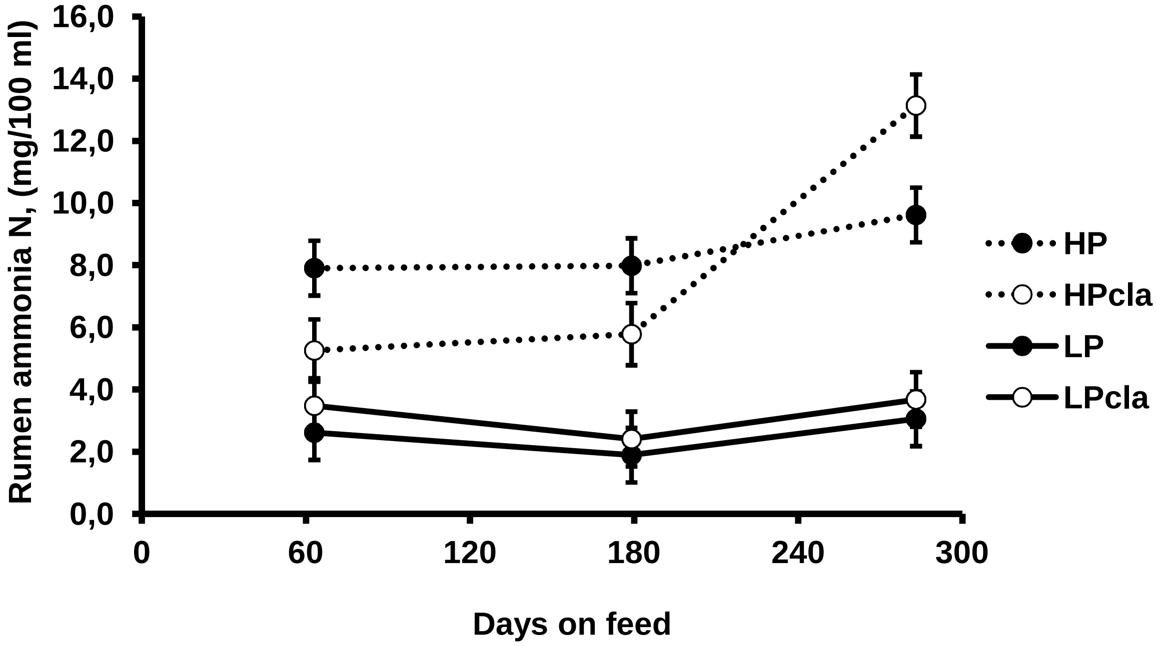


Figure 2. Rumen fluid ammonia N concentrations during the trial as influenced by dietary CP (high CP, HP, dotted lines; low CP, LP, continuos lines) top dressed (○) or not (●) with rumen protected CLA with measurements repeated at 63, 179 and 283 days of feeding. [Data points are the mean of 12 observations; error bars indicate SEM.]



SECOND CONTRIBUTE

Decreasing soybean meal, crude protein and essential amino acids in the diet of finishing heavy pigs fed restricted:

effects on growth performance

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Abstract

This study investigated the effect of decreasing dietary CP and essential amino acids (AA) content relative to conventional diets on growth performance of finishing heavy pigs. Four isoenergetic feed treatments (conventional: CONV; medium-high protein: MHP; medium-low protein: MLP; low protein: LP) were formulated by replacing soybean with wheat meal to contain from 146 to 117 g/kg and from 133 to 108 g/kg CP in early (90 to 130 kg BW) and late finishing (130 to 165 kg BW), respectively. Within period all the diets contained the same amounts of AA per unit of CP. A total of 240 crossbred pigs, grouped in three consecutive batches of 80 pigs each, were assigned to one of the four feed treatments on the basis of BW, sex and litter of origin (10 pigs per pen, 2 pens per treatment) and were fed restricted amount of diets, which increased from 2.4 to 3.2 kg/d from start (92 ± 10 kg BW) to end of trial (167 ± 10 kg BW). For practical applications, N excretion was estimated using a semi-deterministic model as actual N intake – N retention, assuming 0.024 kg N retained/kg BW gain. Comparisons between CONV and low-protein diets were made using Bayesian inference. Average BW at the end of the trial and overall average daily gain and gain to feed ratio (G:F) of pigs fed CONV were close to 168 kg, 0.670 kg/d, and 0.257, respectively. Only MHP pigs showed non-zero differences for final BW and G:F relative to CONV pigs, but the magnitude of differences was small. Final backfat (BF) was thicker for pigs fed MLP and LP diets than for pigs fed CONV, due to an increased average gain in BF. Feed treatments affected the amount of excreted N, which decreased of 10, 20 and 24% for MHP, MLP and LP, respectively, relative to CONV. We conclude that a reduction of CP and AA content of diets for finishing heavy pigs is advisable, as no negative impact on growth performance and feed efficiency was observed with respect to CONV. The inclusion in feeds for heavy pigs of small or null proportion of soybean meal in early and late finishing, respectively, with a minimal supplementation of AA reduces feed costs and N excretion, thus increasing the potential number of pigs per unit of land where the maximum N load/ha is limited by law.

Key words: amino acids content, crude protein content, heavy pigs, growth performance, nitrogen excretion.

Implications

This study evidenced that a reduction of CP and indispensable amino acids (**AA**) content of diets for finishing heavy pigs relative to conventional diets is feasible without any negative impact on growth traits. This can be attained including in feeds for heavy pigs small or null proportion of soybean meal in early and late finishing, respectively, with a minimal supplementation of AA. This favorably impact feed costs, causes a large reduction of N excretion and increases the potential number of pigs that can be reared per unit of land where the maximum N load/ha is limited by law.

Introduction

A reduction of the dietary CP content is an effective way to decrease N excretion of growing pigs (Dourmad and Jondreville, 2007). Based on the ideal protein concept (NRC, 1998), notable reductions of CP can be applied if diets are supplied with essential AA (Otto et al., 2003; Hinson et al., 2009; Hernández et al., 2011).

The pig production system in Italy is dominated by regulation for the production of Protected Designation of Origin (**PDO**) dry-cured ham, which imposes for pigs at slaughter at least 160 kg BW and 9 months of age. Feed restriction is required to match average daily gain (**ADG**) with these targets. Protein and AA needs for finishing heavy pigs have been scarcely defined in experimental studies and commercial feeds are still formulated on the basis of empirical and practical experiences (Mordenti et al., 2003). These feeds contain on average 150 to 130 g CP/kg and 7.0 to 6.5 g lysine (**lys**)/kg feed from 90 to 165 kg BW (BREF, 2003). Previous investigations indicated that in this range of BW protein gain of restricted-fed heavy pigs declines from 108 to 91 g/d (Manini et al., 1997), feed intake increases and the ideal AA profile changes because of the variation of the maintenance to protein retention need ratio.

Our hypothesis is that in such production system concurrent decreases of dietary CP and essential AA content might exert little effects on pig growth performance in the interval 90 to 165 kg BW relative to conventional diets and might lead to decreased N excretion. Moreover, partial replacement of protein feedstuffs with alternative feeds, namely cereal meals, favorably affects pig farming costs. This study aimed to investigate the effect of diets with different CP and essential AA content on growth performance traits. For practical applications, N excretion of heavy pigs estimated using a semi-deterministic model was also considered.

Material and methods

All experimental procedures were reviewed and approved by the Ethical Committee for the Care and Use of Experimental Animals (CEASA) of the University of Padova.

Criteria and methodology for feeds formulation

The control feeds used in early (from 90 to 130 kg BW) and late (from 130 to 170 kg BW) finishing were conventional feeds (**CONV**), representative of those commonly used for Italian heavy pigs for PDO ham production. They provided 12.8 and 12.9 MJ of metabolisable energy (**ME**)/kg, 146 and 133 g CP/kg and 7.6 and 5.9 g lys/kg feed for early and late finishing, respectively. Feed ingredients and chemical composition of the three low-protein treatments were determined as follows. Comparative slaughter experiment performed by Manini et al. (1997) evidenced that in pigs exhibiting 700 g/d ADG, daily protein retention in early and late finishing was on average 100 and 94 g/d, respectively. The NRC (1998) equations were taken as the basis to calculate daily requirements (g/d) of true digestible lys as a function of average metabolic weight ($BW^{0.75}$) and expected daily protein retentions in the two periods (Manini et al., 1997). So, the amount of total lys per day (NRC, 1998) was estimated on the basis of true digestible lys provision. Total lys requirements for early and late finishing were 15.19 and 14.62 g/d, respectively. Dietary total lys concentration for early (6.0 g/kg feed) and late (5.1 g/kg feed) finishing was then determined on the basis of the desired average feed intake (2.53 and 2.87 kg/d for early and late finishing, respectively). As a consequence, for early-finishing feed containing 120 g CP/kg (-18% relative to CONV), the minimum lys concentration is nearly 50 g/kg CP and for late finishing feed containing 110 g CP/kg (-17% relative to CONV) the minimum lys concentration is nearly 46 g/kg CP. These values can be obtained with cereal-based rations providing very low or null amounts of soybean meal with minimal addition of synthetic AA.

Experimental feeds

Three isoenergetic (12.8 and 12.9 MJ of ME/kg in early and late finishing, respectively) low-protein feed treatments were formulated to be compared with CONV. The low-protein feeds were formulated by replacing soybean meal with wheat grain in such a way to provide similar essential AA to CP ratios. These diets were obtained through a 25%- (medium high protein diet, **MHP**), 50%- (medium low protein diet, **MLP**) or 75%-decrease (low protein diet, **LP**) of the amount of soybean meal relative to CONV in early finishing, and through a 40%-, 70%-, or 100%-decrease for MHP, MLP, and LP, respectively, in late finishing. Relative to CONV, the dietary CP content was decreased by 8, 14 or 19% for MHP, MLP or LP treatments, respectively. Small amounts of synthetic AA were added to MHP, MLP, and LP with the aim of maintaining in all diets the same amount of lys, threonine and tryptophan per CP unit. Relative to CONV, lys decrease was 9, 16, and 22% for MHP, MLP, or LP, respectively. A supply of synthetic methionine was not necessary.

Feeds used within a trial and a finishing period were produced using the same batches of feed ingredients. Realized ingredient composition of diets (Table 1), as determined on-line by automated weighing platforms, exhibited trivial differences when compared to the theoretical one. Chemical and nutrient composition of feeds, assessed using chemical analysis or estimated using ingredient composition of feeds and tabular chemical values of each ingredient (Sauvant et al., 2004) is presented in Table 2.

Animals, feeding and experimental design

A total of 240 crossbred pigs (120 gilts and 120 barrows), grouped in three consecutive batches of 80 pigs each, were used in this study. Pigs were offspring of 12 boars of the C21 Goland sire line (Gorzagri, Fonzaso, Italy) mated to 32 Large White-derived crossbred sows. Crossbred sows originated from a cross involving boars of a synthetic line, derived from Large White and Pietrain lines, and sows of a Large White line selected for maternal ability and prolificacy. Besides growth and residual feed efficiency, the breeding goal of the C21 Goland sire line includes traits related to the quality of dry-cured ham. Pigs were born and reared in the same farm and fed a common diet till they were transferred to the experimental farm of the University of Padova.

In each batch, 20 pigs were allotted to one of the four feed treatments on the basis of individual BW, sex, and litter of origin, so that the number of barrows and gilts, of full- and half-sibs, and average BW were similar across feed treatments. Pigs allotted to a feed treatment were housed in two pens of 10 animals each for a total of eight pens. Each pen was 5.8 x 3.8 m, had 40% slatted floor and was equipped with a single-space electronic feeder (Compident Pig – MLP, Schauer Agrotronic, Austria). The equipment recorded for each visit animal identification, the time and date of the feeding event, the amount of feed eaten in that feeding event and the time spent feeding. Water was freely available from a nipple drinker placed in each pen. Pigs were given 10 d to acclimate to feeders before the beginning of the feeding trial. Pigs were fed on the basis of a restricted feeding scale adjusted at 2-wk intervals. Daily amount of pelleted feed per pig ranged from 2.4 to 3.2 kg/d from the first to the last week on feed, when the pigs were expected to weigh 90 and 165 kg BW, respectively. Dietary treatment started at ($\bar{x}$ $\pm s.d.$) 92 $\pm$ 10 kg BW, when pigs were ($\bar{x}$ $\pm s.d.$) 183 $\pm$ 8 d old. Change from early to late finishing diets occurred at ($\bar{x}$ $\pm s.d.$) 132 $\pm$ 11 kg BW, after ($\bar{x}$ $\pm s.d.$) 43 ± 4 d on feed, and each trial had an overall duration of ($\bar{x}$ $\pm s.d.$) 102 ± 8 d.

Individual BW and P2 (above the last rib at approximately 6 cm from midline) backfat depth (**BF**, mm) were measured at 2-week intervals using an electronic scale and an A-mode ultrasonic device (Renco Lean-Meater series 12, Renco corporation, Minneapolis, USA), respectively. Data collected were used to compute individual ADG, average daily feed intake, BF variation and feed efficiency, expressed as gain to feed ratio (**G:F**).

During the trial, seven pigs died or were discarded for respiratory diseases or legs injuries (1 pig for CONV, 3 pigs for MHP, and 3 pigs for MLP). These animals were not considered in the statistical analyses, which included records of 233 pigs.

Feed analysis

During manufacture of feeds provided to each batch of animals 10 samples of each diet were collected on line, pooled, mixed and sampled to achieve a 1-kg feed sample from which independent sub-samples were collected. The feed samples were analyzed in three independent replications for their proximate composition (AOAC, 2003) and NDF content (Van Soest et al., 1991). Starch content was determined, after its hydrolysis to glucose (AOAC, 2003), by liquid chromatography (Bouchard et al., 1988). Metabolizable energy and AA content were assessed from actual ingredient composition of diets and tabular values, according to Sauvant et al. (2004), for each feed ingredient.

Proximate N balance

To assist individual farmers to calculate N excretion of several animal categories, a methodology has been developed within the context of a European Commission funded study (ERM/AB-DLO, 1999; ERM, 2001), which takes into account animal type, diet and other management practices. This methodology, providing institutions and private operators with a framework to calibrate the appropriate stocking rate to comply with constraints imposed by the Nitrates Directive (EEC, 1991), was acknowledged by Italian regulations (MIPAF, 2006). According to this methodology, N excretion was computed in the present study as follows: N intake – N retention, where N intake was computed from feed intake and feed N content, and N retention was estimated from BW gain assuming 0.024 kg N retained/kg BW gain (MIPAF, 2006). Nitrogen efficiency was computed as the ratio between N retention and N intake.

Statistical analyses

Statistical analysis of performance and simulated N balance traits was based on the following linear animal model:

$$y_{ijklmn} = \mu + diet_i + batch_j + sex_k + pen_{l:ij} + dietsire_m + a_{n:ijklm} + (diet \times sex)_{ik} + e_{ijklmn}$$

where y_{ijklmn} is a record for a trait of pig n within diet i, batch j, gender k, pen l, and combined diet-sire class m; μ is the intercept, $diet_i$ is the fixed effect of the feed treatment (i = 1, …, 4), $batch_j$ is the fixed effect of the batch (j = 1, …, 3), sex_k is the fixed effect of gender (k: 1 = barrow, 2 = gilt), $pen_{l:ij}$ is the fixed effect of pen (l = 1, …, 8) within feed treatment and batch, $dietsire_m$ is the random effect of the feed treatment within sire family (m = 1, …, 48), $(diet \times sex)_{ik}$ is the interaction effect between feed treatment and gender, a_n is the random additive genetic effect of the animal (n = 1, …, 233), and e_{ijklmn} is a random residual.

Animal models are extensively used in the field of quantitative genetics to account for infinitesimal genetic effects of individuals.

Bayesian inference was used. All traits were assumed to be conditionally normally distributed as follow:

$$p(\mathbf{y}|\mathbf{b},\mathbf{a}) \sim N(\mathbf{Xb} + \mathbf{Wq} + \mathbf{Za}, \mathbf{I}\sigma_e^2),$$

where **b** was the vector including the fixed effects previously described, **q** was the vector of the random effects of the feeding program within sire family, **a** was the vector of individual additive genetic effects. Bounded uniform priors were used to represent vague previous knowledge of **b**. Prior knowledge on additive genetic effects and effects of the feeding program within sire family was represented by assuming that they were normally distributed, conditionally on the associated variance components, as follows:

$$p(\mathbf{q}|\sigma_q^2) \sim N(\mathbf{0}, \mathbf{I}\sigma_q^2) \text{ and } p(\mathbf{a}|\sigma_a^2) \sim N(\mathbf{0}, \mathbf{A}\sigma_a^2),$$

where **A** was the numerator relationship matrix between individuals, **I** is an identity matrix, and σ_q^2 and σ_a^2 are variance components for effects in **q** and **a**, respectively. The relationship matrix accounted for the additive genetic relationships among animals and was computed on the basis of a pedigree file including all animals with phenotypic records plus 6 generations of ancestors. Marginal posterior distributions of all unknowns were estimated using Gibbs sampling algorithm. After some exploratory analysis we used one chain of 200,000 samples with a burn-in period of 10,000 for each trait. Convergence was tested for each trait using Z criterion of Geweke (1992). Monte Carlo standard error were computed. The mean of the marginal posterior distribution was used as a point estimate of parameters of concern. The marginal posterior distributions of the differences between diet CONV and the other 3 diets were obtained from the Gibbs samples of model solutions. From these distributions, we computed the 95% highest posterior density region for the parameter and the probability for the parameter of being greater (for positive point estimates) or lower (for negative point estimates) than zero. When this probability was greater than 0.9 we considered the parameter estimate different from 0. Details about Bayesian methodology can be found in Blasco (2005) and in Sorensen and Gianola (2002).

Results

Interactions between feed treatments and sex or sire family were negligible for all traits. Discussion for sex and sire effects were not of concern for this study. Hence, only results related to feed treatments are presented and discussed here.

Performance traits

Raw means of performance traits for pigs fed CONV and features of marginal posterior distributions of the difference between restrained-crude protein diets and CONV are presented in Table 3.

Average BW at the end of the trial and overall ADG of pigs fed CONV were close to 168 kg and 0.670 kg/d, respectively. Dietary treatment exhibited limited effect on variation of BW and ADG. A non zero difference between diets was detected only for BW (intermediate and final) and early finishing ADG of MHP. Overall ADG was similar across feeding programs.

Final BF was greater for pigs fed MLP and LP diets than for pigs fed CONV. The estimated difference between MLP or LP and CONV was 0.67 and 0.75 mm for final BF, respectively (i.e., a nearly 4%-increase relative to CONV). This was due to the increased average gain in BF of pigs fed MLP and LP, which was nearly 10% greater relative to that of CONV pigs.

Overall G:F of CONV pigs was 0.257. Pigs fed low-protein diets exhibited trivial differences in feed efficiency relative to CONV pigs. Only MHP pigs showed a non-zero difference for G:F relative to CONV animals, but the magnitude of this estimate was small.

Proximate N balance

Raw means for traits related to proximate N balance of pigs fed CONV and features of marginal posterior distributions of the difference between restrained-crude protein diets and CONV are presented in Table 4.

On average, pigs fed CONV exhibited 57 g/d of overall N intake. Although diet CP content was higher in early than in late finishing, N intake was similar in the two growing phases, because feed intake increased with time. Dietary treatments affected N intake, which was 7, 14 or 17% lower for MHP, MLP, or LP, respectively, than that of CONV. Because feed intake was similar across dietary treatments as a consequence of the restricted feeding system, the decrease in N intake was due to differences in CP content across diets. Proximate daily N retention averaged 16 g/d (i.e., 100 g/d of daily protein retention). The average overall amount of excreted N of CONV pigs was 41 g/d. Feed treatments affected the amount of excreted N. Relative to CONV, the decrease in N excretion was 10, 20 and 24% for MHP, MLP and LP, respectively.

Nitrogen efficiency decreased from early (0.319) to late finishing (0.249), and averaged 0.279 for CONV pigs in the whole trial. Nitrogen efficiency was influenced by feed treatments and increased when dietary CP concentration decreased. Average overall N efficiency was 0.297, 0.325 and 0.338 for MHP, MLP and LP pigs, respectively.

Discussion

In this study Bayesian procedures were used to analyze the data and inferences were based on the marginal posterior probability of non-zero differences between three restrained CP diets and CONV for Italian heavy pigs. Major advantages of these techniques are an increased accuracy when describing uncertainty about the true value of a parameter and the construction of different types of probability intervals to answer different questions (Blasco, 2005).

Dietary CP and growth performance

Performance traits shown by pigs fed CONV were consistent with typical performance required to heavy Italian pigs (Bosi and Russo, 2004; Xiccato et al., 2005). Effects of low CP diets, designed to progressively decrease CP and essential AA content, need to be discussed within the production circumstances dictated by PDO regulations.

Pigs kept under not limiting feeding conditions. Under unlimited feed energy availability and not limiting environmental conditions, body protein gain depends upon the supply of ideal protein available for growth, which should not exceed the requirement of protein for potential growth rate as defined by genetic merit and physiological maturity of the pig (Ferguson et al., 1994). Ideal protein is defined as the protein exhibiting optimal ratios between essential AA in relation to the needs of the pig (NRC, 1998). Needs for maintenance and protein growth affect composition of ideal protein (NRC, 1998). Increases in BW affect composition of ideal protein because the needs for maintenance increase, whereas those for protein growth increase up to a maximum and decline thereafter, going to zero when the animals reach mature protein weight. The concept of ideal protein has important implications. Many studies evidenced that a decrease in the range from 30 to 40 g CP/kg feed and an adequate supply of essential amino acids did not affect growth performance and feed efficiency of pigs fed *ad libitum* up to 100 or 120 kg BW relative to conventional high protein feeds (Kerr et al., 1995; Kerr at al., 2003; Hinson et al., 2009). Conversely, low-protein diets unsupplemented with essential AA decreased ADG and feed efficiency with respect to control feeds or low protein feeds supplemented with essential AA, when pigs were fed *ad libitum* up 100 or 110 kg BW (Kerr et al., 1995; Ruusunen et al., 2007).

Pigs kept under restricted feeding conditions. Feed restriction is commonly applied to constrain ADG to a given target. However, because energy and nutrients are used by the pig with a given order of priority, first for maintenance, than for protein gain and ultimately for fat gain (Ferguson et al., 1994; Mordenti et al., 2003; Sandberg et al., 2005), energy or protein restriction or both may exert different effects on ADG and body composition. In the heavy pig production system, energy restriction is commonly used to constrain ADG in the range 0.65 to 0.7 kg/d. Little attention has been paid to the provision of dietary protein and to its composition for BW greater than 90 to 100 kg (Bosi and Russo, 2004). The rationale of previous investigations for formulating low-protein diets for heavy pigs was greatly influenced by findings of feeding experiments on pigs kept under unlimited feeding conditions until 120 kg final BW. A common behavior was to increase the synthetic AA supplementation when decreasing the dietary CP content. From 80 to 140 and from 140 to 165 kg BW, conventional feeds for heavy pigs provide on average 160 to 140 and 140 to 130 g CP/kg, 7.5 to 6.5 and 7 to 6 g lysine/kg, respectively (BREF, 2003). For heavy pigs fed restricted in the interval 80 to 173 kg BW, Galassi et al. (2010) detected that growth performance and feed efficiency were unaffected by 2 diets providing 120 or 99 g CP/kg feed when diets supplied high amounts of essential AA (6.5, 2.1, 4.5, and 1.4 g/kg feed of lys, methionine, threonine and triptophane, respectively). On the basis of comparative slaughter experiment results Manini et al. (1997) suggested that a content of 5.7 g lys/kg feed is adequate for heavy pigs of 110 kg BW or more. Corino et al. (2008) reported that diets containing 146 g/kg CP and 5.5 or 6.8 g/kg lys did not affect final BW or ADG of pigs from 105 to 153 kg BW, suggesting that 5.5 g lys/kg is an acceptable provision for heavy pigs.

Quantification of optimal dietary CP and AA provision requires the concurrent definition of the amount of feed and ME to provide growing pigs with to achieve the desired ADG and gain composition (Mordenti et al., 2003). Such quantification is made difficult by the fact that pigs with high potential for lean deposition at a given maturity status exhibit enhanced ADG when provided with high dietary CP and AA supply. Conversely, trivial effects on gain performance are expected when the same CP and AA supply is administered to pigs of moderate potential for lean gain (Ferguson et al., 1994; Bosi and Russo, 2004).

In our study diets with the lowest CP content were formulated to meet CP and AA requirements for maintenance and to achieve daily protein gains of 100 and 94 g/d in early and late finishing, respectively. Because no change in ADG and G:F was observed when supply of CP and AA increased, provision of 117 g CP/kg and 5.8 g lysine/kg in early finishing and 108 g CP/kg and 4.6 g lysine/kg in late finishing seems to be adequate.

Results of studies on the effect of dietary CP on carcass composition are controversial. Increased BF or carcass fatness was observed in pigs until 120 kg BW fed *ad libitum* low protein diets with no AA supplementation relative to pigs fed control diets (Kerr at al., 1995; Ruusunen et al., 2007). Conversely, provision of low-CP AA-supplemented diets resulted in no effect (Hinson et al., 2009) or in an increase in BF (Kerr at al., 1995; Tuitoek et al., 1997) relative to control diets. In heavy pigs fed restricted till 170 kg BW, a decrease in dietary CP with AA supplementation was not associated with increased BF (Galassi et al., 2010).

However, restricted dietary lys provision increased BF as a result of decreased protein retention and increased energy availability for fat deposition (Main et al., 2008; Rodríguez-Sánchez et al., 2011).

In our study a decrease of CP and AA dietary content was associated with an increase in BF compared to CONV, which may favorably affect processing ability of carcasses (Bosi and Russo, 2004). Because the amount and quality of subcutaneous fat are critical features for processing ability of raw hams, further investigations are needed to achieve a better insight into the effect of low CP and AA diets on carcass composition.

Proximate N balance

As expected, the decrease of CP dietary content had large effects on the results of proximate N balance.

Nitrogen intake of CONV pigs averaged 57 g/d, a value similar to the 55 g/d of N intake reported by Galassi et al. (2010) for PDO heavy pigs. Knowledge on N retention of heavy pigs in the 80 to 165 kg BW interval is scarce. Nitrogen retention of 21 g/d was observed in metabolic studies based on measurement of feed intake, orts, feces and urine (Galassi et al. 2010). Nitrogen retention assessed in comparative slaughtering trials was 17.3 and 14.6 g/d for 80 and 160 kg BW, respectively. (Manini et al., 1997). Similar values were reported by Shirali et al. (2012) as measured by the deuterium dilution technique in the range from 120 to 140 kg BW. Such inconsistency can be partly ascribed to biased measurements of intake and outputs, which can cause overestimates of N retention (Spanghero et al., 1997). Differences in estimated N retention can also be attributed to use of different feeding regimes, diets, genetic lines, genders and pigs of variable maturity status (Ferguson et al., 1994).

In agreement with Manini et al (1997) and Shirali et al. (2012), N retention estimated in this study averaged 18.2 and 14.5 g/d in early and finishing period, respectively. Overall, the estimated N retention averaged 16 g/d, corresponding to a daily protein retention of 100 g/d. Such estimates need to be discussed in relation to the realized growth performance, the expected empty gain composition and the intake of ME available for protein and fat deposition. Literature data for the interval 60 to 140 kg BW indicate that the water to protein ratio is steady and approximates 2.8 (Just, 1984), ash is 20% of retained protein (Just, 1984), and empty weight gain is approximately 94% of ADG (NRC, 1998). As a consequence, the realized average weight gain (667 g/d) can be partitioned in 400 g/d fat free mass (100 g/d protein + 20 g/d ash + 280 g/d water) and 227 g/d fat (627 g empty weight gain − 400 g fat free mass). According to NRC (1998), ME requirements for protein and fat deposition are 44.37 and 52.32 MJ/kg, respectively. Hence, the required ME to support protein and lipid gain of 100 and 227 g/d, respectively, is 16.34 MJ/d. The realized feed intake in the trial averaged 2.595 kg/d (i.e., 33.5 MJ/d ME intake). Because average $BW^{0.75}$ was 38.35 kg, the estimated ME used for maintenance and growth were 17.00 MJ/d and 16.33 MJ/d, respectively. These values are consistent with the ME required to support the estimated protein and fat deposition.

These results indicate that average protein retention during the trial was consistent with values assumed to formulate LP and that the average N retention of 0.024 kg/kg BW gain assumed by the Italian regulation on manure disposal (MIPAF, 2006) can be considered realistic for fed-restricted heavy pigs in the range 80 to 170 kg BW. This has been recently confirmed by Guo et al. (2012).

Because N excretion is primarily influenced by N intake (Cahn et al., 1998), biased quantification of true N retention per kg BW gain has trivial consequences for the estimation of N excretion. Shirali et al. (2012) reported estimated correlations between daily N intake and daily N excretion of 0.93 and 0.96 in the range 90 to 120 and 120 to 140 kg BW, respectively. In agreement with Cahn et al. (1998) and Galassi et al. (2010), results of our study indicate that a 10-g/kg decrease in dietary CP is associated with a 9% decrease in N excretion, and the replacement of CONV with LP reduces N excretion of about 24%.

In conclusion, data from this study evidenced that diets containing 117 and 108 g CP/kg and 5.8 and 4.6 lysine/kg are adequate to support growth performance of restricted-fed heavy pigs in the range 90 to 130 and 130 to 165 kg BW, respectively. These amounts are noticeably lower than those currently provided by conventional feeds in the same BW range, and can be attained through an almost complete replacement of soybean meal with wheat meal and a minimal supplementation of essential AA. This strategy impacts the profitability of herds by decreasing feeding costs and by reducing N excretion, thus increasing the potential number of pigs that can be reared per year and per unit of agricultural land in agreement with the Italian regulation for manure disposal. Further studies are needed to investigate the effects of low CP and AA diets on carcass composition and ham quality.

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Table 1. *Ingredient composition (g/kg as fed) of early (90 to 130 kg average BW) and late finisher diets (over 130 kg average BW)*

	Early finisher diets[1]				Late finisher diets			
	CONV	MHP	MLP	LP	CONV	MHP	MLP	LP
Ingredient[2]:								
Corn grain	347.9	347.0	344.3	347.3	367.2	356.7	357.4	359.9
Barley grain	198.9	201.9	197.8	199.1	200.0	199.6	199.5	200.4
Wheat grain	76.6	112.1	148.1	177.6	104.9	155.8	184.8	204.5
Soybean meal	133.3	99.9	62.9	29.4	94.8	55.4	25.5	0.00
Wheat bran	120.3	117.2	120.8	123.0	125.2	124.2	124.6	126.2
Wheat middlings	59.2	58.6	61.7	59.1	50.2	49.5	49.6	49.9
Cane molasses	20.3	19.9	20.1	20.2	20.0	20.2	19.9	20.0
Beef tallow	15.3	14.0	13.5	12.6	14.2	13.3	12.3	11.3
Calcium carbonate	14.6	15.0	15.2	15.6	13.5	13.9	13.9	13.9
Dicalcium phosphate	4.2	4.6	4.6	4.4	2.1	2.2	2.2	2.7
Sodium bicarbonate	2.9	2.6	2.6	2.6	2.5	2.6	2.5	2.7
Sodium chloride	2.9	2.9	2.9	2.9	3.0	3.0	3.0	3.0
Vit. and min.premix[3]	1.9	1.9	1.9	1.9	2.0	2.0	2.0	2.0
Choline HCl	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
L-Lysine HCl	1.3	2.0	2.9	3.3	0.0	1.0	1.9	2.5
L-Threonine	0.0	0.0	0.3	0.5	0.0	0.2	0.4	0.5
L-Tryptophan	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1

[1] CONV, conventional diet, high protein content; MHP: medium-high protein diet; MLP: medium-low protein diet; LP: low protein diet.

[2] Actual daily loads of feed ingredients recorded by the weighing platforms of the feed firm. Data are the means of three batches.

[3] Providing per kg of complete diet: vitamin A 7.500 UI, vitamin D_3 1.600 UI; vitamin B_1 1,4 mg; vitamin B_2 3,5 mg; vitamin B_6 2,5 mg; vitamin B_{12} 0,02 mg; vitamin E 40 mg; vitamin K_3 1,75 mg; vitamin PP 25 mg; pantothenic acid 18 mg; folic acid 0,6 mg; biotin 0,25 mg; choline 350 mg; manganese 45 mg, zinc 70 mg, iron 90 mg, copper 10 mg, iodine 1,5 mg, selenium 0,3 mg.

Table 2. *Nutrient content (g/kg as fed, unless otherwise indicated) of early (90 to 130 kg average BW) and late finisher diets (over 130 kg average BW)*

	Early finisher diets [1]				Late finisher diets			
	CONV	MHP	MLP	LP	CONV	MHP	MLP	LP
Analyzed nutrient composition[2]*:*								
DM	881	880	878	883	885	885	884	884
CP (N × 6.25)	146	135	127	117	133	121	112	108
Starch	426	434	451	455	436	454	470	476
NDF	129	130	135	137	133	134	133	131
Ether extract	40	39	40	38	40	39	38	36
Ash	47	46	45	44	43	42	41	40
Calculated nutrient composition [3]								
ME, MJ/kg	12.8	12.8	12.8	12.8	12.9	12.9	12.9	12.9
Lysine	7.6	7.0	6.4	5.8	5.9	5.3	5.0	4.6
Methionine	2.4	2.3	2.1	1.9	2.2	2.0	1.9	1.8
Methionine + Cystine	5.3	4.9	4.6	4.2	4.9	4.6	4.3	4.1
Threonine	5.5	4.9	4.6	4.2	4.9	4.5	4.2	3.9
Tryptophan	1.8	1.7	1.5	1.4	1.6	1.4	1.4	1.3

[1] CONV, conventional diet, high protein content; MHP: medium-high protein diet; MLP: medium-low protein diet ; LP: low protein diet.

[2]Analytical results obtained according to AOAC (2003) by averaging data on 3 independent replications.

[3] According to Sauvant et al. (2004).

Table 3. *Raw mean of pigs fed the conventional diets (CONV mean) and features of the marginal posterior distributions of the differences between diets for performance traits (N = 233 pigs)*[1]

Item	CONV Mean	MHP vs CONV Estimate[2]	MHP vs CONV P0[3]	MHP vs CONV HPD95[4]	MLP vs CONV Estimate	MLP vs CONV P0	MLP vs CONV HPD95	LP vs CONV Estimate	LP vs CONV P0	LP vs CONV HPD95
BW, kg:										
initial	92.5	-1.3	0.74	-5.5; 2.8	-1.8	0.78	-6.4; 2.7	0.2	0.53	-4.3; 4.6
intermediate	130.0	- 3.1	0.94	-7.0; 0.7	-0.6	0.61	-5.1; 3.2	0.4	0.57	-4.0; 4.7
final	168.4	-2.6	0.90	-6.8; 1.5	-2.0	0.80	-6.9; 2.8	1.4	0.72	-3.4; 6.2
Average daily gain, kg/d:										
early finishing [5]	0.757	-0.035	0.91	-0.088; 0.016	0.022	0.77	-0.035; 0.081	0.000	0.50	-0.055; 0.056
late finishing [6]	0.605	0.001	0.68	-0.031; 0.051	-0.024	0.86	-0.067; 0.020	0.012	0.71	-0.031; 0.054
overall	0.667	-0.001	0.68	-0.045; 0.027	-0.004	0.57	-0.042; 0.036	0.009	0.67	-0.030; 0.047
Backfat thickness, mm:										
initial	9.97	-0.28	0.84	-0.84; 0.28	-0.22	0.77	-0.82; 0.38	-0.11	0.65	-0.69; 0.50
intermediate	12.63	-0.45	0.92	-1.1; 0.17	0.55	0.95	-0.12; 1.22	0.18	0.70	-0.49; 0.84
final	17.52	0.05	0.53	-1.01; 1.15	0.67	0.87	-0.50; 1.87	0.75	0.90	-0.38; 1.94
Average gain in backfat, mm										
early finishing	2.66	-0.13	0.67	-0.71; 0.46	0.78	0.99	0.15; 1.42	0.23	0.76	-0.41; 0.84
late finishing	4.47	0.56	0.90	-0.29; 1.41	0.13	0.61	-0.79; 1.06	0.61	0.91	-0.28; 1.53
overall	7.55	0.34	0.74	-0.68; 1.39	0.84	0.93	-0.28; 1.97	0.76	0.91	-0.34; 1.85
Gain to feed ratio, kg/kg										
early finishing period	0.310	-0.013	0.94	-0.029; 0.003	0.006	0.75	-0.012; 0.024	-0.001	0.54	-0.018; 0.017
late finishing period	0.220	-0.003	0.73	-0.015; 0.008	-0.011	0.96	-0.022; 0.001	-0.004	0.78	-0.016; 0.007
overall	0.257	-0.008	0.94	-0.017; 0.003	-0.004	0.77	-0.014; 0.006	-0.005	0.82	-0.015; 0.005

[1] CONV, conventional diet, high protein content; MHP: medium-high protein diet; MLP: medium-low protein diet ; LP: low protein diet.

[2]Estimated differences between solutions of MHP or MLP or LP and CONV diet.

[3]Marginal posterior probability of the estimate of being greater (for positive estimate) or lower (for negative estimates) than 0.

[4]Lower and upper bounds of the 95% highest posterior density region.

[5]43 ± 4 days on feed.

[6]59 ± 4 days on feed.

Table 4. *Raw mean of pigs fed the conventional diets (CONV mean) and features of the marginal posterior distributions of the differences between diets for proximate N balance (N = 233 pigs)*[1,2]

		MHP vs. C			MLP vs. C			LP vs C		
Item:	CONV Mean	Estimate[3]	P0[4]	HPD95[5]	Estimate	P0	HPD95	Estimate	P0	HPD95
N intake, g/d:										
early finishing [6]	56.6	- 5.2	1	-6.8; -3.6	-8.7	1	-10.3; - 7.0	-11.0	1	-12.7; -9.2
late finishing [7]	58.0	-3.3	1	-4.2; -1.9	- 7.7	1	-9.5; -5.9	-8.8	1	-10.3; -7.2
overall	57.3	-4.1	1	-5.4; -2.9	-8.3	1	-9.6; -6.8	-9.6	1	-11.0; -8.1
N retention, g/d:										
early finishing	18.2	-0.84	0.90	-2.08; 0.40	0.53	0.78	-0.80; 1.87	0.01	0.50	-1.33; 1.41
late finishing	14.5	0.25	0.69	-0.75; 1.24	-0.58	0.87	-1.61; 0.45	0.30	0.72	-0.72; 1.32
overall	16.0	-0.21	0.68	-1.06; 0.64	-0.07	0.57	-1.00; 0.86	0.22	0.68	-0.69; 1.15
N excretion, g/d:										
early finishing	38.4	-4.35	1	-5.19; -3.51	-8.35	1	-9.24; -7.43	-11.04	1	-11.93; -10.16
late finishing	43.5	-3.57	1	-4.53; -2.62	-8.11	1	-9.14; -7.05	-9.11	1	-10.14; -8.09
overall	41.3	-3.94	1	-4.65; -3.23	-8.26	1	-9.00; -7.49	-9.84	1	-10.58; -9.07
N efficiency[8], kg/kg										
early finishing	0.319	0.012	0.90	-0.007; 0.032	0.063	1	0.042; 0.084	0.079	1	0.058; 0.099
late finishing	0.249	0.020	0.99	0.005; 0.035	0.031	1	0.015; 0.046	0.052	1	0.037; 0.068
- overall	0.279	0.018	1	0.005; 0.030	0.046	1	0.033; 0.059	0.061	1	0.049; 0.074

[1] CONV, conventional diet, high protein content; MHP: medium-high protein diet; MLP: medium-low protein diet ; LP: low protein diet.

[2]Computed according to the methodology proposed by ERM (2001) to assist individual farmers to calculate N in slurry.

[3]Estimated differences between solutions of MHP or MLP or LP and CONV diet.

[4]Marginal posterior probability of the estimate of being greater (for positive estimate) or lower (for negative estimates) than 0.

[5]Lower and upper bounds of high posterior density interval at 95% of probability.

[6]43 ± 4. days on feed.

[7]59 ± 4. days on feed.

[8]Computed as N retention/N intake.

THIRD CONTRIBUTE

Effects of a progressive reductions of dietary CP and essential amino acids contents on carcass traits and ham quality of fed-restricted heavy pigs for dry-cured ham production

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Unpublished

Abstract

This study investigated the effect of decreasing dietary CP and essential amino acids (AA) content relative to conventional diets on carcass traits and ham quality of finishing heavy pigs. Four isoenergetic feed treatments (conventional: CONV; medium-high protein: MHP; medium-low protein: MLP; low protein: LP) were formulated by replacing soybean with wheat meal to contain from 146 to 117 g/kg and from 133 to 108 g/kg CP in early (90 to 130 kg BW) and late finishing (130 to 165 kg BW), respectively. Within period all the diets contained the same amounts of AA per unit of CP. A total of 240 crossbred pigs, grouped in three consecutive batches of 80 pigs each, were assigned to one of the four feed treatments on the basis of BW, sex and litter of origin (10 pigs per pen, 2 pens per treatment) and were fed restricted amount of diets, which increased from 2.4 to 3.2 kg/d from start (92 ± 10 kg BW) to end of trial (167 ± 10 kg BW). During the trial, seven pigs died or were discarded for respiratory diseases or legs injuries, so only 233 animals were considered in this experiment. All the pigs were slaughtered in the same day at 286 ± 1.9 days old in a commercial abattoir. After slaughter, hot carcass weight was recorded and the carcass backfat depth (mm) was measured with a millimeter rule on the points of maximum and minimum thickness. The carcass was jointed. The following lean and fat cuts were separated from the two semi-carcass and weighted. The percentages of lean and fat cuts were computed with respect to weight of the hot carcass. After 24-h of chilling, fresh hams were trimmed according to procedure the typical-shape of San Daniele ham. After trimming, hams were weighed and ham fat covering was measured by an ultrasound device at the internal part of depth in correspondence of semimembranosus muscle and by a millimeter-stick at the external part of the ham in correspondence of biceps femoris. The trimmed fat was collected to be analyzed for iodine number and for fatty acid composition. The day after, the trimmed hams were transferred to the processing firm were all the hams were processed according to the San Daniele ham procedure involving the following phases: salting (16 ± 1 d), resting (98 ± 5 d) and seasoning (239 ± 24 d). At the beginning and the start of each processing phase the hams were weighed. Percentage losses in weight were expressed with respect to the weight of trimmed ham at beginning of salting. There were no signs of influence of the dietary treatment on the weights of the various lean cuts. With respect to CONV the treatment with the lowest dietary CP increased significantly the weight of the fat cuts and their proportion on carcass weight from 0.238 to 0.244. The reduction of the dietary CP content significantly increased ham fat covering from 20.4 to 23.3 mm and reduced total weight loss of ham from 0.285 to 0.275. The results of this experiment indicates that dietary CP contents in the early and finishing period of 117 and 108 g CP/kg respectively, do not influence negatively the growth performance and slightly improves the quality characteristics of hams. We conclude that the use of low protein diets over 90 kg BW is strongly suggested as a strategy to reduce the feeding costs, to reduce the N emission and the land surface required for manure spreading and to improve the quality characteristics of hams.

Key words*:* Pig, dry-cured ham, carcass, ham quality.

Introduction

Cured ham is a traditional product of great economic importance for the meat industry in the Mediterranean area and of increasing interest in other parts of the world (Ramos et al., 2007). In Italy the dry-cured ham industry for the production of Protected Designation of Origin (PDO) hams requires pigs slaughtered at body weight (BW) in the range of 150-170 kg at least 9 months old with carcass weights in the range of 125-140 kg (Guo et al., 2012), fresh hams with a minimum weight of 12 kg, with a minimum fat covering of 15 mm (to avoid excessive weight losses during processing) and with a maximum of 0.15 and 70 of linoleic acid on total fatty acids and iodine number, respectively, in the fat that cover the ham, to minimize oxidation and rancidity during curing (PDO San Daniele Ham, 1996). Ham represents over 0.55 of total commercial value of the carcass, even if it represent only 0.18-0.20 of carcass mass (Russo et al., 1990; Chizzolini et al., 1995). To achieved these targets feeding regime and diet characteristics must be properly formulated. In the recent decade increasing costs of protein sources and synthetic amino acids, and the need of reducing nitrogen emission from piggery, have strongly increased the interest for using diets with low amounts of these constituents, particularly in the range of 90 to 170 kg BW. Previous researches evidenced that a reduction of dietary CP with addition of essential amino acids had little consequences on growth performance and carcass traits of light pigs (Whittemore et al., 2001; Tagliapietra et al., 2005; Ringel and Susenbeth, 2009), however no information are available about the effect of diets with low CP and low essential AA on carcass traits and ham characteristics when applied on pigs over 90 kg BW. A recent work, were dietary CP was progressively reduced from 146 to 117 g/kg and from 133 to 108 g/kg in early (90 to 130 kg BW) and late finishing (130 to 165 kg BW), respectively, with a proportional decrease in essential amino acids, evidenced that with respect to control diets with the lowest CP content did not impaired growth performance and feed efficiency of pigs in the range from 90 to 165 kg BW (Gallo et al., unpublished). Aim of this paper was to evaluate, on the same pigs used by Gallo et al. (unpublished), the effects of diets with decreasing amounts of CP and essential amino acids on carcass traits and of fresh ham characteristics and weight losses during the dry curing process.

Material and Methods

All experimental procedures were reviewed and approved by Ethical Committee for the Care and Use of Experimental Animals (CEASA) of the University of Padova. The reader is referred to the parallel paper of Gallo et al. (unpublished, second contribute of this thesis) for major details about this experiment.

Animals, feeding regime and experimental design

A total of 240 crossbred pigs (120 gilts and 120 barrows), grouped in three consecutive batches of 80 pigs each, were used. Pigs were offspring of 12 boars of the C21 Goland sire line (Gorzagri, Fanzoso, Italy) mated to 32 Large White-derived crossbred sows. Crossbred sows originated from a cross involving boars of a synthetic line, derived from Large White and Pietrain lines, and sows of a Large White line selected for maternal ability and prolificacy. Besides growth and residual feed efficiency, the breeding goal of the C21 Goland sire line includes traits related to the quality of dry-cured ham. Pigs were born and reared in the same farm and fed a common diet till they were transferred to the experimental farm of the University of Padova. In each batch, 20 pigs were allotted to one of four feed treatments on the basis of individual BW, sex, and litter of origin, so that the number of barrows and gilts, of full- and half-sibs, and average BW were similar across feed treatments. Pigs allotted to a feed treatment were housed in two pens of 10 animals each for a total of eight pens. Each pen was 5.8 × 3.8 m, had 40% slatted floor and was equipped with a single-space electronic feeder (Compident Pig – MLP, Schauer Agrotronic, Austria). The equipment recorded for each visit animal identification, the time and date of the feeding event, the amount of feed eaten in that feeding event and the time spent feeding. Water was freely available from a nipple drinker placed in each pen. Pigs were given 10 d to acclimate to feeders before the beginning of the feeding trial. Pigs were fed on the basis of a restricted feeding scale adjusted at 2-wk intervals. Daily amount of pelleted feed per pig ranged from 2.4 to 3.2 kg/d from the first to the last week on feed, when the pigs were expected to weigh 90 and 165 kg BW, respectively. Dietary treatment started at $(\bar{x} \pm \mathrm{s.d.})\ 92 \pm 10\ \mathrm{kg\ BW}$, when pigs were $(\bar{x} \pm \mathrm{s.d.})\ 183 \pm 8\ \mathrm{d\ old}$. Change from early to late finishing diets occurred at $(\bar{x} \pm$

s.d.) 132 ± 11 kg BW, after ($\bar{x}$ ± s.d.) 43 ± 4 d on feed, and each trial had an overall duration of ($\bar{x}$ ± s.d.) 102 ± 8 d.

Feeding treatments

The control feeds used in early (from 90 to 130 kg BW) and late (from 130 to 170 kg BW) finishing were conventional feeds (CONV), representative of those commonly used for italian heavy pigs for PDO ham production. They provided 12.8 and 12.9 MJ of metabolisable energy (ME)/kg, 146 and 133 g CP/kg and 7.6 and 5.9 g lysine/kg feed for early and late finishing, respectively. Three isoenergetic (12.8 and 12.9 MJ of ME/kg in early and late finishing, respectively) low-protein feed treatments were formulated to be compared with CONV. The low-protein feeds were formulated by replacing soybean meal with wheat grain in such a way to provide similar essential AA to CP ratios. These diets were obtained through a 25%- (medium high protein diet, MHP), 50%- (medium low protein diet, MLP) or 75%-decrease (low protein diet, LP) of the amount of soybean meal relative to CONV in early finishing, and through a 40%-, 70%-, or 100%-decrease for MHP, MLP and LP, respectively, in late finishing. Relative to CONV, the dietary CP content was decreased by 8, 14, or 19% for MHP, MLP or LP treatments, respectively. Small amounts of syntetic AA were added to MHP, MLP and LP with the aim of maintaining in all diets the same amount of lysine, threonine and tryptophan per CP unit. Relative to CONV, lysine decrease was 9, 16, and 22% for MHP, MLP or LP, respectively. A supply of synthetic methionine was not necessary.
Feeds used within trial and finishing period were produced using the same batches of feed ingredients. Actual ingredient and chemical composition of diets are presented in Table 1 and Table 2, respectively. Major details about criteria for feed formulation and chemical analysis are given in Gallo et al. (unpublished, second contribute of this thesis).

Slaughtering and dissection procedures

During the trial, seven pigs died or were discarded for respiratory diseases or legs injuries (1 pig for CONV, 3 pigs for MHP, and 3 pigs for MLP). So only 233 animals were considered in this experiment. All the pigs were slaughtered in the same day at 286 ± 1.9 days old (average BW of 167.6 ± 1.8 kg) in a commercial abattoir (Salumificio UANETTO, Castions di Strada, Udine, Italy), according to the abattoir procedures. After slaughter, hot carcass weight was recorded and the carcass backfat depth (mm) was measured with a millimeter rule on the points of maximum and minimum thickness. The carcass was jointed. The following lean and fat cuts were separated from the two semi-carcass and weighted according to the Modena

procedures described by Russo (1989): namely, loin, shoulder, ham and neck, belly, backfat and collar fat.
The percentages of lean and fat cuts were computed with respect to weight of the hot carcass.

Measurements of ham characteristics

After 24-h of chilling, fresh hams were trimmed according to procedure the typical-shape of San Daniele ham. After trimming, hams were weighed and two measurements of the thickness of fat covering were recorded. Ham fat covering was measured by an ultrasound device at the internal part of depth in correspondence of semimembranosus muscle and by a millimeter-stick at the external part of the ham in correspondence of biceps femoris. The trimmed fat was collected to be analyzed for iodine number according to Wijs (# 920.259; AOAC, 2000) and for fatty acid composition according to (Folch et al., 1957) by gas-chromatography (GC 17A, Shimadzu, Kyoto, Japan).
The day after, the trimmed hams were transferred to the processing firm (La Casa del Prosciutto, San Daniele del Friuli, Udine, Italy) were all the hams were processed according to the San Daniele ham procedure (DOP Prosciutto San Daniele, 1996) involving the following phases: salting (16 ± 1 d), resting (98 ± 5 d) and seasoning (239 ± 24 d). At the beginning and the start of each processing phase the hams were weighed. Percentage losses in weight were expressed with respect to the weight of trimmed ham at beginning of salting.

Statistical analyses

Data were analyzed using PROC GLM of SAS (2005). Statistical analyses of carcass traits and crude-dry ham quality was based on the following linear animal model:

$$y_{ijklmn} = \mu + diet_i + batch_j + sex_k + pen_{l:ij} + diet\text{-}sire_m + a_{n:ijklm} + (diet \times sex)_{ik} + e_{ijklmn}$$

where y_{ijklmn} is an experimental observation; μ is the overall mean, $diet_i$ is the fixed effect of the feed treatment (i = 1, …, 4), $batch_j$ is the fixed effect of the batch (j = 1, …, 3), sex_k is the fixed effect of gender (k: 1 = barrow, 2 = gilt), $pen_{l:ij}$ is the fixed effect of pen (l = 1, …, 8) within feed treatment and batch, $diet\text{-}sire_m$ is the random effect of the feed treatment within sire family (m = 1, …, 48), $(diet \times sex)_{ik}$ is the interaction effect between feed treatment and gender, a_n is the random additive genetic effect of the animal (n = 1, …, 233), and e_{ijklmn} is a random residual.

Results

Carcass traits

The reduction of dietary CP and amino acids content tended to increased (P = 0.05) carcass weight, but there were no effects of the masses of lean cuts, loin, neck, shoulder and ham (Table 3). Differently, the reduction of CP and AA increased significantly (P = 0.049) the mass of fat cuts, particularly that of collar fat (P <0.01). The percentage of lean cuts on carcass weight was unaffected by the dietary CP and AA density, whereas the percentage of fat cuts was increased when lower amounts of CP and AA were supplied (P = 0.043).

Sex influenced carcass weight (P=0.012), neck (P=0.015), weight of fat cuts (P=0.025) in particular collar fat (P < 0.01) and backfat (P < 0.01), and carcass backfat thickness at the point of minimum depth (P<0.01). No significant effect due to diet × sex interaction were detected.

Trimmed hams characteristics

Compared to CONV, the LP treatment did not changed the weight of trimmed hams, however, for MHP and MLP treatments the weight of hams was significantly reduced compared to control and LP (Table 4).

The thickness of ham covering fat, measured at the external part of the ham, was increased by about 15% with MLP and LP compared with CONV and MHP (P=0.006), whereas the fat thickness measured in the internal part of the ham was not influenced by the dietary CP and AA content (P =0.15).

Some influence of sex was found for the minimum fat depth (P<0.001) measured at the internal part of the ham, but no significant effect due to diet × sex interaction were observed.

Fatty acid composition of fresh ham fat samples

No significant effect due to diet, sex and their interaction was observed on the iodine number, however the percentage of linoleic acid decreased progressively from 13.13 to 12.51% of total FA passing from CONV to LP (Table 5). There were no effect of the dietary treatment on the percentage of SFA, but a reduction of dietary CP and AA content increased the percentage of MUFA and decreased the percentage of PUFA, so that the (MUFA + PUFA)/SFA was not influenced by the dietary treatment. The feeding treatment had also some effects on the amount of Ω-3 and Ω-6 fatty acids.

Sex influence only MUFA (P=0.014), and no significant effect due to diet × sex interaction were observed.

Weight and weight losses during the dry cured hams processing

The weight of ham at the beginning of salting was comparable to that observed for the trimmed ham at the slaughter house, with a significant effect due to the feeding treatment (Table 6). After salting, resting and seasoning there were no significant differences due to the feeding treatment on the weight of hams, although CONV and LP tended to shown heavier ham compared to MHP and MLP ($P = 0.05$).

The dietary treatment did not influenced the ham weight losses during salting, but strongly influenced the ham weight losses during resting ($P < 0.001$) and seasoning ($P< 0.001$). So at the end of the dry-curing process the ham weight losses were about 1 percentage point less for MLP and LP compared to HP and MHP.

No significant effect due to sex and diet × sex interaction were observed on these parameters.

Discussion

Carcass traits

The average value for the various carcass traits considered in the current experiment can be compared with some values given in literature for heavy pigs in the DOP dry-cured ham circuit. Around 160 to 170 kg of slaughter BW dressing percentage ranged from 0.80 to 0.82 and carcass weight was frequently between 130 to 140 kg (Corino et al., 2003; Bosi and Russo, 2004; Lo Fiego et al. 2005; Corino et al., 2008; Della Casa et al. 2010). These data are in agreement with the result of current experiment where BW at slaughter (Gallo et al. unpublished, second contribute of this thesis) averaged 168 kg, carcass weight averaged 138 kg and dressing percentage averaged 81.6. Ham yield, as proportion of warm carcass weight, was frequently found in the order of 0.24 to 0.25 (Della Casa et al. 2010), trimmed ham yield was in the order of 0.20 (Corino et al. 2003) and in the current experiment these values averaged 0.24 and 0.22, respectively. To our knowledge little or no data about the weight of others lean and fat cuts of 160-170 kg BW heavy pigs are given in literature. Data from the current experiment indicated that 138-kg carcass contains on average 84.3 kg of lean cuts (0.616 as proportion of warm carcass weight), of which 25.4, 9.2, 16.6 and 33.4 kg of loin, neck, shoulder and ham, respectively, and 33.1 kg of fat cuts (0.241 as proportion of carcass weight; flare fat was not collected), of which 7.6, 17.3 and 8.2 of collar fat, belly and backfat, respectively.

In the current experiment, where the all the pigs received the same amounts of feeds over the growing period, there were no signs of influence of the dietary treatment on the weights of the various lean cuts. This suggest that in the range of live weight under observation the amount of CP and essential AA supplied with LP was sufficient to meet the requirement for maintenance and protein growth. Based on the NRC (1998) framework, the LP diets were formulated to provide 15.2 and 14.6 g lysine/d to support maintenance and an assumed protein growth of 100 and 94 g/d in the early and finishing period. The lack of effects of the feeding treatment on the weights of the various lean cuts suggest that, with respect to the LP diets, higher amount of dietary lysine, other essential AA and CP were likely exceeding the requirements.

However, with respect to CONV the treatment with the lowest dietary CP increased significantly the weight of the fat cuts and their proportion on carcass weight from 0.238 to 0.244. In absolute terms this difference is small corresponding to about 1 kg of fat cuts. From a theoretical point of view such result would have been expected as the replacement of soybean (CP) with cereals (Starch) would have increased the use efficiency of ME, or in other terms, the dietary content of net energy (Mordenti et al., 2003).

Ham quality

Because the dry-curing process last at least 12 months, studies regarding the relationships between measurements performed on carcass and fresh hams and those regarding the final quality of the dry cured hams are very important from the economical point of view, even though the knowledge of such relationships is still inadequate, because of the cost associated with these studies. A number of pre-slaughtering and post-slaughtering factors affect the quality of dry-cured hams (Bosi and Russo, 2004). Besides factors such the genetic type of the pigs and the feeding characteristics, those which are considered to exerts major influences are: the lean percentage of carcass, which is negatively related to the weight losses of hams during processing (Nanni Costa et al., 1990; Russo et al., 1990), the weight of trimmed fresh hams, which must have a minimum value of 12 kg (DOP Prosciutto San Daniele, 1996), the fat covering which should be at least 15 mm thick, with an optimal range of values between 20 and 30 mm (Lo Fiego et al., 2005). Consortia supervising the production of DOP hams have also fixed thresholds for the iodine value, which must not exceed 70, and the content of linoleic acid (C18:2), which must not to be greater than 0.15 of total fatty acids.

The weight loss of hams during processing is considered an indirect index of the final quality of hams. Weight loss of ham during processing is a measure of the dehydration degree and of salt absorption which inhibit microbial proliferation and permits the correct maturation of the products, however from the economical point of view it also represent a cost because it reduces the weight of saleable product (Russo et al., 1990). Ham weight losses during curing commonly ranges between 0.20 and over 0.30 (Diaferia et al., 1994). A deep fat cover improves the attitude of the meat to salting and curing, due to the fact that covering fat contains less water than muscular tissue, thus reducing exchanges between muscle and the external environment (Carnier et al., 1999; Gallo et al., 1999; Candek-Potokar et al., 2002).

In agreement with these authors, in the current experiment the reduction of the dietary CP content significantly increased ham fat covering from 20.4 to 23.3 mm and reduced total weight loss of ham from 0.285 to 0.275. Fat covering and total weight losses were correlated, but weakly (Figure 1; $R^2 = 0.23$). The results of the current experiment also indicated that the almost complete replacement of soybean meal with wheat grain, even if not sufficient to reduce the iodine value, reduced slightly the percentage of linoleic acid from 0.131 to 0.125. Overall the result of this experiment indicates that dietary CP contents in the early and finishing period of 117 and 108 g CP/kg (providing 15.2 and 14.6 g lysine/d), respectively, do not influence negatively the growth performance and slightly improves the quality characteristics of hams. Thus, the use of low protein diets over 90 kg BW is strongly suggested as a strategy to reduce the feeding costs, to reduce the N emission and the land surface required for manure spreading and to improve the quality characteristics of hams. Finally it must be considered that such results were obtained on a genetic line of pigs which has received a genetic selection for ham quality, with a moderate emphasis on lean growth. Further studies are required to evaluate the effect of such strategy on pigs of different genetic lines.

Conclusions

This study evidenced as a reduction of CP and indispensable amino acids (AA) content of diets for finishing heavy pigs relative to conventional diets can be used without negative impact on carcass traits and with an improvement of dry cured hams quality in term of greater fat covering, lower linoleic acid content and lower total weight losses during processing compared to conventional diets. As from a previous experiment conducted with the same pigs is was found that the use of a low-protein diet does not impair growth performance but reduces strongly N emission and the feeding costs, such strategy is recommended to improve the economical and the environmental sustainability and the quality the products in the heavy pig system for dry cured ham production.

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Table 1. *Ingredient composition (g/kg as fed) of early (90 to 130 kg average BW) and late finisher diets (over 130 kg average BW).*

	Early finisher diets[4]				Late finished diets[5]			
	CONV[1]	MHP	MLP	LP	CONV	MHP	MLP	LP
Ingredients[2]:								
Corn grain	347.9	347.0	344.3	347.3	367.2	356.7	357.4	359.9
Barley grain	198.9	201.9	197.8	199.1	200.0	199.6	199.5	200.4
Wheat grain	76.6	112.1	148.1	177.6	104.9	155.8	184.8	204.5
Soybean meal	133.3	99.9	62.9	29.4	94.8	55.4	25.5	0.0
Wheat bran	120.3	117.2	120.8	123.0	125.2	124.2	124.6	126.2
Wheat middlings	59.2	58.6	61.7	59.1	50.2	49.5	49.6	49.9
Cane molasses	20.3	19.9	20.1	20.2	20.0	20.2	19.9	20.0
Beef tallow	15.3	14.0	13.5	12.6	14.2	13.3	12.3	11.3
Calcium carbonate	14.6	15.0	15.2	15.6	13.5	13.9	13.9	13.9
Dicalcium phosphate	4.2	4.6	4.6	4.4	2.1	2.2	2.2	2.7
Sodium bicarbonate	2.9	2.6	2.6	2.6	2.5	2.6	2.5	2.7
Sodium chloride	2.9	2.9	2.9	2.9	3.0	3.0	3.0	3.0
Vit. and min. premix[3]	1.9	1.9	1.9	1.9	2.0	2.0	2.0	2.0
Choline HCl	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
L-Lysine HCl	1.3	2.0	2.9	3.3	0.0	1.0	1.9	2.5
L-Threonine	0.0	0.0	0.3	0.5	0.0	0.2	0.4	0.5
L-Tryptophan	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1

[1] CONV: conventional diet, high protein content; MHP: medium-high protein diet; MLP: medium-low protein diet; LP: low protein diet. [2] Actual daily loads of feed ingredients recorded by the weighing platforms of the feed firm. Data are means batches.

[2] Actual daily loads of feed ingredients recorded by the weighing platforms of the feed firm. Data are the means of three batches.

[3] Providing per kg of complete diet: vitamin A 7.500 UI, vitamin D_3 1.600 UI; vitamin B_1 1,4 mg; vitamin B_2 3,5 mg; vitamin B_6 2,5 mg; vitamin B_{12} 0,02 mg; vitamin E 40 mg; vitamin K_3 1,75 mg; vitamin PP 25 mg; pantothenic acid 18 mg; folic acid 0,6 mg; biotin 0,25 mg; choline 350 mg; manganese 45 mg, zinc 70 mg, iron 90 mg, copper 10 mg, iodine 1,5 mg, selenium 0,3 mg.

[4] 43 ± 4 days on feed.

[5] 59 ± 4 days on feed.

Table 2. Nutrient content (g/kg as fed, unless otherwise indicated) of early (90 to 130 kg average BW) and finisher diets (over 130 kg average BW).

	Early finisher diets[4]				Late finished diets[5]			
	CONV[1]	MHP	MLP	LP	CONV	MHP	MLP	LP
Analyzed nutrient composition[2]:								
DM	881	880	878	883	885	885	884	884
CP (N x 6.25)	146	135	127	117	133	121	112	108
Starch	426	434	451	455	436	454	470	476
NDF	129	130	135	137	133	134	133	131
Ether extract	40	39	40	38	40	39	38	36
Ash	47	46	45	44	43	42	41	40
Calculated nutrient composition[3]:								
ME, MJ/kg	12.8	12.8	12.8	12.8	12.9	12.9	12.9	12.9
Lysine	7.6	7.0	6.4	5.8	5.9	5.3	5.0	4.6
Methionine	2.4	2.3	2.1	1.9	2.2	2.0	1.9	1.8
Methionine + Cystine	5.3	4.9	4.6	4.2	4.9	4.6	4.3	4.1
Threonine	5.5	4.9	4.6	4.2	4.9	4.5	4.2	3.9
Tryptophan	1.8	1.7	1.5	1.4	1.6	1.4	1.4	1.3

[1] CONV: conventional diet, high protein content; MHP: medium-high protein diet; MLP: medium-low protein diet; LP: low protein diet.

[2] Analytical results obtained according to AOAC (2003) by averaging data on 3 independent replications.

[3] According to Sauvant et al. (2004).

[4] 43 ± 4 days on feed.

[5] 59 ± 4 days on feed.

Table 3. Carcass traits of heavy pigs fed diets with decreasing CP and essential amino acids contents[2].

	Treatment[1]				SEM			
	CONV	MHP	MLP	LP		Diet	Sex	Diet x Sex
Carcass weight, kg	137.8	134.9	136.7	139.0	0.55	0.05	0.012	0.84
Lean cuts, kg:	84.9	83.1	84.6	84.6	0.50	0.52	0.21	0.57
Loin	25.4	24.7	26.1	25.3	0.36	0.69	0.37	0.42
Neck	9.1	9.0	9.0	9.2	0.04	0.13	0.015	0.22
Shoulder	16.6	16.4	16.5	16.7	0.10	0.39	0.06	0.72
Ham	33.8	33.1	33.0	33.8	0.16	0.09	0.93	0.86
Fat cuts, kg:	32.8	32.2	33.3	33.9	0.27	0.049	0.025	0.88
Collar fat	7.5	7.4	7.6	7.8	0.05	0.005	<0.001	0.94
Belly	17.3	16.9	17.3	17.8	0.16	0.05	0.63	0.54
Backfat	8.1	7.9	8.4	8.3	0.10	0.22	0.004	0.82
Lean cuts, % of carcass	61.5	61.6	61.9	61.2	0.00	0.88	0.50	0.69
Fat cuts, % of carcass	23.8	23.7	24.5	24.4	0.00	0.043	0.10	0.69
Carcass backfat depht, mm								
at point of maximum depth	38.3	37.8	40.0	39.0	0.37	0.32	0.38	0.76
at point of minimum depth	23.3	23.7	24.2	24.0	0.30	0.76	0.003	0.68

[1] CONV: conventional diet, high protein content; MHP: medium-high protein diet; MLP: medium-low protein diet; LP: low protein diet.

[2] Data are about 233 pigs that at slaughter were 286 ± 1.9 days old.

Table 4. Trimmed cold hams traits of heavy pigs fed diets with decreasing CP and essential amino acids contents[4].

	Treatment[1]				SEM			
	CONV	MHP	MLP	LP		Diet	Sex	Diet x Sex
Trimmed cold hams:								
Weight, kg	30.1	29.4	29.4	30.2	0.14	0.045	0.91	0.82
Maximum fat depth, mm[2]	20.4	20.8	24.0	23.3	0.41	0.006	0.30	0.40
Minimum fat depth, mm[3]	5.6	5.9	5.9	5.7	0.00	0.15	<0.001	0.58

[1] CONV: conventional diet, high protein content; MHP: medium-high protein diet; MLP: medium-low protein diet; LP: low protein diet.

[2] Measured with millimetre-stick at the external part of the ham in correspondence of biceps femoris.

[3] Measured with ultrasound device at the internal part of ham in correspondance of semimembranosus muscle.

[4] Data are about 233 pigs that at slaughter were 286 ± 1.9 days old. The weights of the two hams obtained from the same pig were summed.

Table 5. Weight and weight losses during processing of heavy pigs fed diets with decreasing CP and essential amino acids contents[5].

	Treatment[1]				SEM			
	CONV	MHP	MLP	LP		Diet	Sex	Diet x Sex
Hams weight, kg:								
Start of salting	29.6	28.9	28.8	29.6	0.13	0.041	0.78	0.70
End of salting[2]	28.6	27.9	28.0	28.7	0.13	0.045	0.81	0.60
End of resting[3]	24.1	23.6	23.7	23.3	0.11	0.06	0.90	0.47
End of seasoning[4]	21.1	20.7	20.9	21.4	0.11	0.05	0.81	0.47
Hams weight losses:								
End of salting, %	3.35	3.32	3.21	3.17	0.039	0.28	0.67	0.09
End of resting, %	18.39	18.26	17.77	17.73	0.101	0.009	0.10	0.18
End of seasoning, %	28.50	28.46	27.56	27.54	0.133	0.004	0.17	0.41

[1] CONV: conventional diet, high protein content; MHP: medium-high protein diet; MLP: medium-low protein diet; LP: low protein diet.

[2] 16 ± 1 days duration of salting phase.

[3] 98 ± 5 days duration of resting phase.

[4] 239 ± 24 days duration of seasoning phase.

[5] Data are about 233 pigs that at slaughter were 286 ± 1.9 days old. Data from the two hams obtained from the same pig were summed in the case of weights and averaged in the case of weight losses.

Table 6. Analyzed fatty composition of ham trimmed fat (Iodine number[2] and fatty acid profile[3]) of heavy pigs fed diets with decreasing CP and essential amino acids contents.

	Treatment[1]				SEM			
	CONV	MHP	MLP	LP		Diet	Sex	Diet x Sex
Iodine number	65.3	65.2	65.5	64.9	0.20	0.80	0.91	0.26
Linoleic acid	13.13	13.10	12.66	12.51	0.091	0.013	0.23	0.69
SFA[4]	36.95	37.01	36.95	37.29	0.108	0.64	0.38	0.16
MUFA[5]	46.60	47.07	47.62	47.30	0.104	0.012	0.014	0.36
PUFA[6]	14.21	14.16	13.68	13.52	0.085	0.013	0.27	0.73
Ω-3	0.88	0.85	0.81	0.82	0.005	<0.001	0.94	0.41
Ω-6	12.52	12.50	12.05	11.89	0.078	0.013	0.22	0.69
(MUFA + PUFA)/SFA	1.65	1.66	1.66	1.63	0.007	0.49	0.18	0.10
MUFA/PUFA	3.30	3.37	3.52	3.51	0.024	0.006	0.08	0.63

[1] CONV: conventional diet, high protein content; MHP: medium-high protein diet; MLP: medium-low protein diet; LP: low protein diet.

[2] According to Wijs procedure (# 920.259; AOAC, 2000)

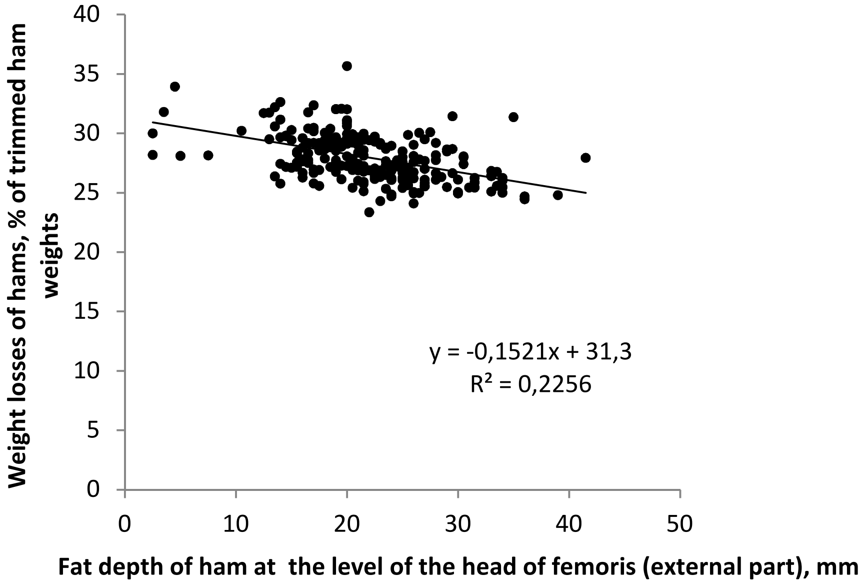
[3] Using gascromathographic analysis (Folch et al., 1957).

[4] Saturated Fatty Acids

[5] MonoUnsaturated Fatty Acids

[6] PolyUnsaturated Fatty Acids

Figure 1. Relationship between fat depth of the external part of ham with total weight losses during processing.



GENERAL CONCLUSIONS

In FIRST CONTRIBUTE it was evidenced that in DBM Piemontese bulls the dietary CP content can be lowered from 145 to 108 g/kg without negative consequences on growth performance, energy and N retention, but with a strong reduction (30%) of N excretion. Compared to conventional diets, feeding low-protein diets does not influence the digestibility of feed constituents but markedly improves N efficiency. Besides this, as during the last 3 months of the growing period average daily gain decreased whereas DM intake increased, an anticipation of the age at slaughter, i.e. the replacement of older subject with younger and more efficient bulls, would strongly reduce N excretion and increase the number of bull reared and meat production per unit of land surface. These results were obtained on DBM Piemontese bulls, which are considered to require dietary CP densities higher than conventional bulls because of their high ability for lean growth and low feed intake. Thus, it could be hypothesized that such strategy would be valid also for conventional bulls, and further experiment are required to support this hypothesis. In addition it was found that both low CP diets and rumen protected CLA supplementation positively affected N efficiency, supporting the hypothesis that rumen protected CLA exerts some protein-sparing effect, but only when dietary CP is low. From an economic aspect, use of rumen protected CLA for a long time at the dose employed in our experiment is unlikely. Further research employing lower doses and/or shorter periods of administration is suggested.

The SECOND CONTRIBUTE evidenced that diets containing 117 and 108 g CP/kg and 5.8 and 4.6 lysine/kg are adequate to support growth performance of restricted-fed heavy pigs in the range 90 to 130 and 130 to 165 kg BW, respectively. These amounts are noticeably lower than those currently provided by conventional feeds in the same BW range, and can be attained through an almost complete replacement of soybean meal with wheat meal and a minimal supplementation of essential AA. This strategy impacts the profitability of herds by decreasing feeding costs and by reducing N excretion, thus increasing the potential number of pigs that can be reared per year and per unit of agricultural land in agreement with the Italian regulation for manure disposal.

The THIRD CONTRIBUTE evidenced that diets containing 117 and 108 g CP/kg and 5.8 and 4.6 lysine/kg in early (90-120 kg BW) and finishing (120 to 165 kg BW) periods do not impact on the quality of carcass traits, but slightly improves the quality of dry cured hams in term of thicker fat covering, lower linoleic acid content and lower total weight losses during processing compared to conventional diets.

The overall results of this thesis permits to conclude that the use of low-protein diets represent a valid strategy, at least in DBM bulls and heavy pigs, to conjugate the needs of a technical, environmental and economic sustainability of meat industry. The results obtained in this thesis would be useful for the scientific community, for the public Institutions and for the producers and the operators in this sector. Both in bulls and in heavy pigs it was found that good performance and good quality of products can be achieved with an almost or total replacement of soybean meal with no protein sources. Our country, as well as other countries of the European Union, is not self-sufficient for this crop. Thus further environmental and economic benefits generated from the application of these feeding strategies remain to be evaluated. Studies conducted at territorial level would be very appreciated.