Milk Ketone Bodies Assessment in A Local Italian Cow Breed (Modenese) vs. Holstein and Characterization of its Physiological, Reproductive and Productive Performances

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Abstract— *In Northern Italy are present several autochthonous cattle breeds characterized by a small territorial diffusion. These breeds were breed for century the milk and meat production but also for work. The technical data show that these animals have a good reproductive performances and disease resistance and resilience. The objective of this study was to characterize some productive, reproductive and metabolic parameters (ketone bodies) in the Italian autochthonous cattle breeds Modenese, comparing them with those of Holstein and crossbred (F1=Modenese x Holstein; F2=Modenese x F1) breed in the same farm in order to understand if there is a different metabolic situation that can influence the reproductive performances. The milk samples have been taken at different times of lactation. Milk samples have been collected from the whole udder during morning milking and from a lactometer and analyzed by Gas chromatography-mass spectrometry to obtain the ketone bodies concentration. The reproductive (Open Days Period and number of Services Per Pregnancy) and productive (percentage and kg of protein between the 40th and 90th DIM) data have been recovered by the consultation of the farm registers and the APA (Provincial breeder association) data. On days open, number of services per pregnancy, % of proteins in milk, and kg of proteins in milk, a Spearman correlation analysis was applied. The comparative study between the races in the same breeding conditions is a fundamental tool to understand the correct reproductive and productive physiology of dairy cattle. Among the ketone bodies concentration, the Modenese breed showed a significant ($p<0,05$) lower concentration. A high concentration of ketone bodies is related with serious negative energy balance condition that can affect the reproductive performances. The F1, F2 and Modenese showed also better reproductive performances compared to Holstein, with a day's open length in average between the 80-105 days. In conclusion, the better resilience against the negative energy balance and his adverse effects of Modenese cattle could be one of the phenomena underlying their better reproductive efficiency.*

Keywords— Italian autochthonous cattle breeds, dairy cows, ketone bodies, reproduction, production.

I. INTRODUCTION

In the last 60 years the bovine genetic selection has been focused only on the milk production criterion, neglecting the metabolic and muscularity aspects; the Holstein, Brown and Jersey are proof. This mono-aptitude selective criterion has caused a decline in several aspects, the main ones being reproductive performances (*Washburn, S. P. et al 2002, Royal, M. D., et al. 2002, Lucy, M. C. 2001, Esposito et al 2014*) and disease resistance and resilience (metabolic syndrome, ketosis, mastitis and foot diseases) *(Roxström A. et al., 2001a; Roxström A. et al., 2001b, Sørensen A.C. et al.2007, Carlén E. et al 2004)*. The higher production of milk does not correspond to a real gain for the farmer, because the metabolic problems, reproductive disorders and inflammatory problems are increasing both the direct costs, such as the costs for veterinary and medicinal products, and the indirect costs, influencing in a negative way the lactation curve (*Cools S. et al . 2008*) and the economic gain.

An adequate reproductive performance of the lactating herd is a major component of profitability in dairy farms *(Ribeiro E.S. et al., 2012)*. Reproduction is the essential physiological stage to obtain a higher milk production efficiency. Without the effect of the pregnancy and calving it is not possible to reach an optimal development of mammary system and milk production. The scientific studies have identified the optimal cow lactation period around 305 days and the calving to conception interval (days open) around 85 days (*Hafez, B., & Hafez, E. S. E., 2000*). These physiological data are far from situation of the modern dairy farm where more than 50% of cows exceed the 305 days (*Steri R. et al 2012*, *Vargas B. et al., 2000; González-Recio O. et al., 2006; Cole J.B. et al., 2009*) and the days open period is around the 150 days (official national analysis Italian Breeder Association AIA, 2014).

The lengthening of these physiological times depends on many factors that affect the post-partum period, where about 50% of the cows suffer from metabolic or inflammatory disorders that influence the reproductive performance (*LeBlanc S., 2010*). During the days from calving to conception (days open period) are diagnosed the 75% of the diseases that can be found throughout the whole productive life of the cow (*LeBlanc S., 2010*) such as the retained placenta, metritis, endometritis, lameness, puerperal collapse, hypomagnesemia, fatty liver, ketosis, abomasal displacement left and right, hypoplasia ovarian and ovarian cysts. Many of these diseases have as trigger the negative energy balance (NEB) typical of the early lactation period (first 4-6 weeks) caused by the high energy demand required for the milk production associated with the physiological suppression of appetite *(Santos J. E. P. et al., 2014)*. The energy balance, in addition to predispose to many disease, causes also a direct effect on the reproductive performance in the cows *(Butler WR., 2003)*. During the NEB we have an increase of the NEFA and the beta-hydroxybutyrate (BHBA). The NEFA high concentration (≥0,7 mM) reduces the resume of the ovarian cyclicity before the 50 days *(Ribeiro ES. et al 2013)*, while the elevated BHBA concentration are negatively associated with the probability of pregnancy after the first postpartum artificial insemination *(Walsh RB. et al 2007)*. Another marker of NEB is the milk protein percentage. As showed by Fulkerson et al. (2001), the cows with low milk protein percentage suffered from more severe and prolonged NEB compared to cows with higher milk protein percentage. Milk protein percentage has been reported to be positively associated with cow fertility, the cows with a higher milk protein percentage in early lactation have a better reproductive performance (*Yang L. et al 2009, Morton J.M. et al 2001*).

Most of the studies about the reproductive disorders are focused on the cosmopolitan breeds, Holstein and Brown Swiss, because they are one of the major reported reasons for culling in dairy herds with lameness and udder health (*Waiblinger S. et al. 2004; Wathes RB. et al. 2007; Cozler YL.et al. 2009*). Analyzing the Italian autochthonous cattle breed, we found a totally different situation. Some studies have demonstrated that these animals have an early reactivation of the ovarian activity after the calving that permits a precocious insemination with a reduction of the days open period under 100 days *(Communod R. et al 2010)*. Not all the physiological mechanism underlying this phenomenon are known, but as showed by Communod et al (2010), one important factor is the uterine involution. They demonstrated that the Cabannina and Varzese breeds (two local breeds of the northern Italy) have a shorter timeframe for uterine involution compared to the Holstein. These results could explain the early resumption of ovarian activity and the early fecundation opportunity typical of these breeds: in fact, the onset of first detectable estrus can be observed 20 days after birth and the opportunity to impregnate can occur in the following cycle, i.e. approximately 40 from birth *(Communod R. et al 2011)*.

In Italy, a great biodiversity in term of autochthonous cattle breed is present. There are 19 breeds officially recognized and most of them are located in the northern/centre sides of Italy. Before the second world war, these breeds were fundamental for the Italian food production and agriculture, because all of them were characterized by a double (milk and meat) or triple attitude (besides the milk and meat, these animals were used for the work, thanks to their great muscle efficiency). Unfortunately, the animal husbandry of the 21st century has brought a decline in biodiversity of bovine breeds, due to the abandonment of autochthonous cows in favor of more productive cosmopolitan breeds only following the milk production increase goal. Although the reduction of the number of the heads, many autochthonous cattle breeds show interesting characteristics. In the north of the Italy we found the Grey Alpine, the Cabannina, the Varzese, Friuli Red Spotted, Reggiana the Valdostana Red Pied and the Modenese, also called Bianca Val Padana. In our work we were focused on the Modenese.

The objective of this study was to characterize some productive, reproductive and metabolic parameters (ketone bodies) in the Modenese breed, comparing them with those of Holstein breed in the same farm in order to understand if there is a different metabolic situation that can influence the reproductive performances.

II. MATERIALS AND METHODS

Comparison between Holstein and Modenese was carried out in the farm 'Società emplice Agricola Cornetti Alessandro e f.llis.s.' located in Quinzano d'Oglio (CR), the peculiarity of this farm is that it has implemented a plan to replace the entire original Holstein herd with Modenese breed using the crossbreeding methods.

The Bianca Val Padana cattle is also known as "Modenese cattle". It takes its name from the area of distribution around the provinces of Modena, Reggio Emilia, Mantova, Ferrara, Bologna, where it was highly appreciated for its very good milk production. In 1960 there were about 142.000 heads, nowadays their number has greatly decreased because of the highly competitive diffusion of cosmopolitan breeds. Today we have approximately 650 cows of Modenese breed, of which 60% reared in herds together with the Italian Holstein cows ((*Duclos D.R. and Hiemstra S.J., 2010, Petrera F. et al 2014*). According to breed standards, adult female biancavalpadana cows are 125-140 centimeters in height with an average body weight of 650 kilograms. Adult male bulls are 130-160 centimeters in height with an average body weight of 980 kilograms. The females of the breed have all white coats while the males white coats with grey areas on their necks, shoulders, and hips. Another typical characteristic of some Italian white breed is the so-called 'cut', that is a pink coloured reversed V in the centre of the dark grey wide muzzle. Modenese cattle are used for both milk and meat production. The average milk production is 4700 kg in 305 day of lactation with 3.4% of protein and 3.3% of fat (*Duclos D.R. and Hiemstra S.J., 2010*). The milk of Modenese breed is especially suitable for the production of Parmigiano-Reggiano cheese, thanks to its high percentage of casein and its high performance in the cheese-making process.

2.1 Data

The reproductive (Open Days Period and number of Services Per Pregnancy) and productive (percentage and kg of protein between the 40^{th} and 90^{th} DIM) data have been recovered by the consultation of the farm registers and the APA (Provincial breeder association) data.

2.2 Milk samples

A total of 19 animals were sampled for this study:

- 4 Holstein
- 6 Modenese
- 5 F1 (Modenese x Holstein)
- 8 F2 (Modenese x F1)

All these animals were in the second lactation and their calvings were in the same period (within 10 days) and none has undergone treatments in order to obtain the estrus synchronization.

The milk sample have been taken at different time of lactation (T1=20^{th} DIM, T2=40^{th} DIM, T3=90^{th} DIM) between February to April 2014. Milk samples have been collected from the whole udder during morning milking and from a lactometer.

2.3 Milk samples analysis

2.3.1 Reagents and standards

Acetone and cyclohexanone (used as internal standard -IS) were purchased by Sigma (Steinheim, Germany).

2.3.2 Sample preparation and Headspace–Solid-Phase Microextraction (HS-SPME):

All samples were prepared by weighing exactly 5 g of milk in a 20 ml glass vial, fitted with cap equipped with silicon/PTFE septa (Supelco, Bellefonte, PA, USA), and by adding 10 µl of the IS solution in ethanol (0.2 mg ml-1). Standard solutions of acetone in water, containing increasing amounts of analyte (from 2 to 300 µg ml-1) and of 10 µl of IS solution were added to a fresh milk sample to obtain the calibration curve. A temperature of 10°C was selected as extraction and equilibration temperature, in order to prevent possible matrix alteration. To keep the temperature constant during analysis, the vials were maintained in a cooling plate (CTC Analytics, Zwingen, Switzerland). At the end of the sample equilibration time (1 h), a conditioned (1,5 h at 280°C) 85 µm Carboxen/polydimethylsiloxane (CAR/PDMS) StableFlex fibre (Supelco, Bellefonte, PA, USA) was exposed to the headspace of the sample for analyte extraction (15 min) by CombiPAL system injector autosampler (CTC Analytics, Zwingen, Switzerland). Recovery was studied by adding 100 µg ml-1 of acetone to a milks ample and by comparing the results obtained from the sample and the spiked one.

2.3.3 Gas chromatography-mass spectrometry apparatus and conditions:

Analyses were performed with a Trace GC Ultra coupled to a quadrupole mass spectrometer (MS) Trace DSQ (Thermo-Fisher Scientific, Waltham, MA, USA) and equipped with an Rtx-Wax column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) (Restek, Bellefonte, PA, USA).

Oven temperature program was: from 35°C, hold 8 min, to 60 °C at 4 °C min-1, then from 60°C to 160°C at 6°C min-1 and finally from 160 to 200 at 20°C min-1. Helium was the carrier gas, at flow rate of 1 ml min-1. Carry over and peaks originating from the fibre were regularly assessed by running blank samples. After each analysis fibres were immediately thermally desorbed in the GC injector for 5 min at 250°C to prevent contamination.

MS operated in electron impact (EI) ionization mode at 70 eV. Ion source temperature was 250°C. Selected ion monitoring (SIM) was used as data acquisition mode, the ions chosen being 43 and 58 for acetone and 55 and 98 for cyclohexanone, respectively. The dwell time was set to 100 ms. Identification of acetone and IS was carried out by comparing GC retention time with those of standard compounds.Concentration of acetone was determined in triplicate as relative area (analyte area/IS area) (Ra) using the calibration curve.

2.4 Data analysis

Ketone bodies concentrations mean values over the three periods were evaluated with a two-way ANOVA, considering the period and the year as fixed factors.

On days open, number of services per pregnancy, % of proteins in milk, and kg of proteins in milk, a Spearman correlation analysis was applied. The statistical significance was set at $p<0.05$.

III. RESULTS AND DISCUSSION

3.1 Ketone Bodies (Holstein Vs Modenese)

Analyzing the data obtained regarding the values of the ketone bodies in three different time of lactation (20 days, 40 days, 90 days) concerning only the pure breeds Holstein and Modenese, it was found that the lactation period has never been significant for the concentration of ketone bodies, while there is an increasing trend in the samples taken at 40 days from calving (figure 1). This period coincides with the beginning of the peak of lactation (*Hafez, B., & Hafez, E. S. E., 2000*). This tendency is valid for all three ketone bodies.

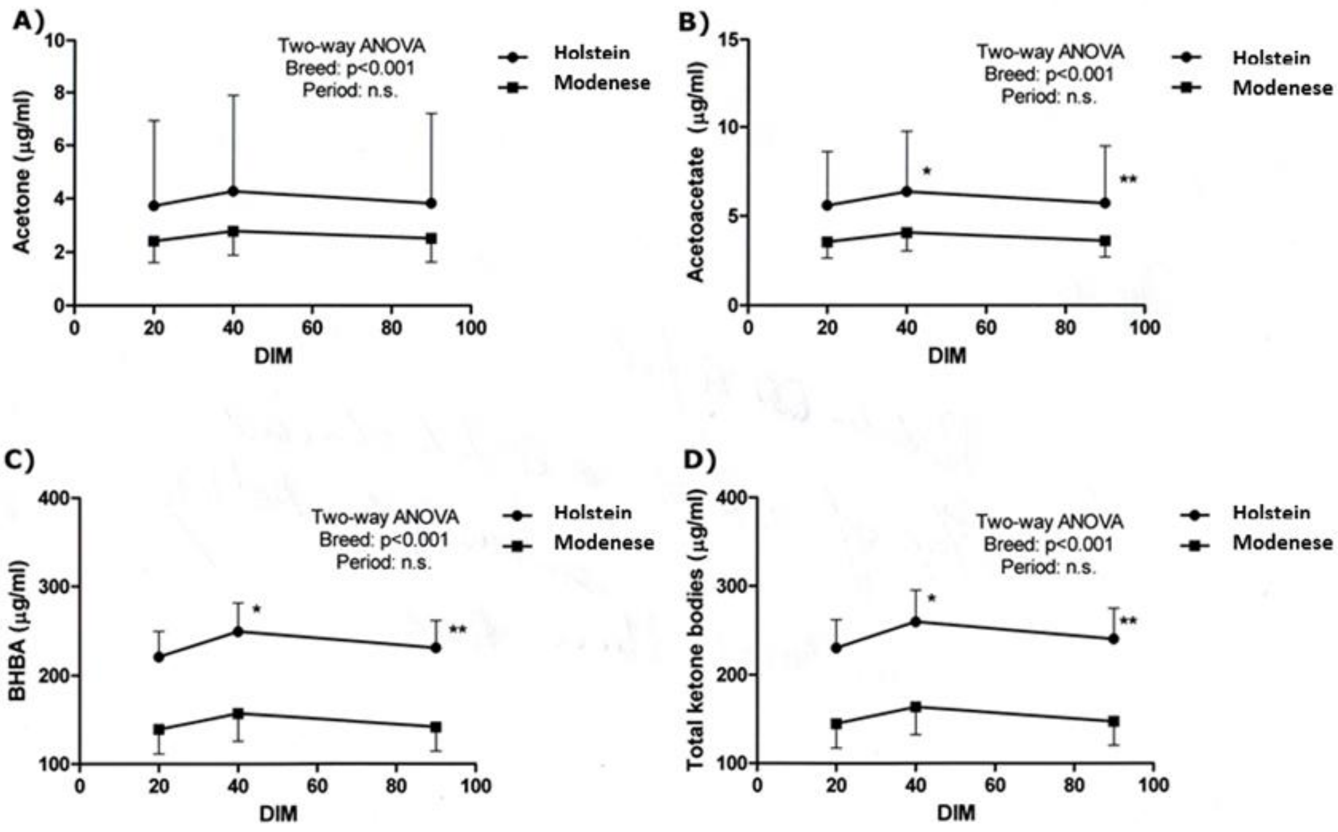

FIGURE 1: THE FIGURE SHOW THE GRAPHICS RELATIVE TO THE COMPARISON OF THE KETONE BODIES MILK CONCENTRATION IN TWO GROUP OF ANIMALS, MODENESE VS HOLSTEIN, THIS DATA ARE REFERRED TO THREE DIFFERENT TIME OF LACTATION (20 DAYS, 40 DAYS, 90 DAYS). *- p<0-05***

Comparing the ketone bodies concentration in the three periods (20th,40th,90th days in milk) (Figure 1) between different genetic lines (Holstein against the Modenese) there are big statistically significant differences ($p < 0.05$) for the acetoacetate and BHBA at the 40th and 90th days in milk (DIM) while, for the acetone the difference founded isn't statistically significant. The explanation of these results could depend on two factors. The first factor, as it is easy to find in the literature, is the higher production of the Holstein that causes many difficulties to the energy metabolism, in particular the carbohydrate metabolism (*Veerkamp R.F. et al., 2003*). The second factor could be referring to the fact that the Holstein are characterized by a smaller amount of muscles. The muscular system, especially the skeletal muscle, is physiologically able to oxidize the ketone bodies (*Schaff C. et al. 2013, Brennan K.M. et al. 2009*) and to make bioavailable a larger quantity of glycogen as well as to release a big amount of amino acids that can best support the neoglucogenesis (*Van Der Drif SG. et al. 2012*). Therefore, the muscular system has an important role in the reduction of the intensity of the NEB and in the mitigation of the negative effects of the ketone bodies released during the NEB period. The perfect energetic metabolism of the Modenese breed (as showed by the ketone bodies concentration) is essential to ensure good reproduction efficiency, since it allows an optimal functioning of the hypothalamic -pituitary -ovarian axis (*Judd, S. J., 1997, Iwata, K. et al 2011*) and the entire female genital system,

3.2 The reproductive and productive data (Modenese, Crossbreed, Holstein)

The first variable analyzed was the Days open (table 1), the results show a statistically significant differences between different genetic lines, and the Modenese/crossbreed have the Days open period significantly lower. For the factor year, there is a statistically significant difference between the year 2013 and the year 2014. The evaluation of the days open period evidences that in F1, F2 and Modenese the length in average is between the 80-105 days, in full compliance with the provisions of the reproductive physiology of dairy cows (*Hafez, B., & Hafez, E. S. E., 2000*) and allow to reach the goal of one calf per year, this goal is impossible in the Holstein were the average of days open is 215±30 days. The good reproductive efficiency of Modenese and crossbreed is also showed by the number of service per pregnancy that is < 2 against the value of 3.35 of the Holstein, this difference is a statistical significant. The factor year, was not statistically relevant.

TABLE 1
MEAN VALUES ± STANDARD ERROR OF THE PARAMETERS EVALUATED IN THE STUDY FOR THE THREE GENETIC GROUPS AND FOR THE YEARS 2013-14. THE TABLE SHOWS THE SIGNIFICANCE STATISTICS FOR THE GROUPS CONSIDERED. SUPERSCRIPTS INDICATE STATISTICAL DIFFERENCES WITH P <0.05 BETWEEN GENETIC GROUPS: DIFFERENT LETTER CORRESPONDS TO A DIFFERENCE AT P < 0.05.

Variable	F1	F2	Modenese	Holstein	2013	2014	Genetic statistical significance	Statistical significance per year
Days open (dd)	99.95 ± 7.81[a]	114.77 ±10.47[a]	89.24 ± 12.51[a]	215±30[b]	147.7 ±15	125 ± 21.5	0.17	0.025
Number of Service Per Pregnancy	1.73 ± 0.12[a]	1.63 ± 0.16[a]	1.70 ± 0.19[a]	3.35±0.2[b]	3.1± 1.7	3.3 ± 2.1	0.82	0.16
% protein	3.25 ± 0.04[b]	3.44 ± 0.05[a]	3.38 ± 0.06[ab]	3.35±0.1[b]	3.15 ± 0.03	3.32 ±0.14	0.0011	0.23
Kg prot (kg)	50.80 ± 1.[a]	38.08 ±2.23[b]	32.24 ± 2.66[b]	41.33±10.40[ab]	40.37 ±1.58	43.31 ± 1.39	<0.001	0.14

The productive parameters analyzed were the milk proteins percentage and kilograms. For the percentage of milk proteins we found a statistically significant difference between the averages of the different genetic lines, the F2 have percentages of protein significantly higher than the Holstein and F1, while the Modenese have an intermediate value. The year does not influence this parameter. The milk protein percentage is an important indicator for both the energy balance and the reproductive efficiency (*Yang, L. et al 2010*). Elevate milk production is associated with reduction of the glucose blood levels, and glucose shortage would result in a reduction of synthesis of milk protein; thus, low milk protein percentage would be found in cows with high milk production and low fertility (*Yang, L. et al 2010*). The percentage of protein in the milk arrives at levels of excellence in particular regarding to the generation F2, values that are even higher than the purebred Modenese. This finding could be explained as a phenomenon of the synergy of the cross between F1 and pure breed.

Finally, we analyzed the kg of proteins produced, that has emerged to be influenced in a statistically significant manner by the different genetic lines. The factor year, also in this variable, has not been shown statistically relevant. The analysis of kg of milk protein produced show that the F1 have the higher values, this is correlatable to their higher milk production (about a 30 % higher) due to the greater influence of the generation progenitor (Holstein).

We analyzed also the correlation between productive/reproductive parameters (table 1). We found a positive correlation between the days open and number of service per pregnancy (SPP) only in the F1 and in the pure breed Modenese. This result is justified by the fact that a longer days open means a higher number of artificial fecundation to obtain a pregnancy. In the Modenese we found a negative correlation between the milk protein percentage and the number of service per pregnancy, this result seems to confirm that the cows with an higher protein percentage during first part of lactation (between the 40-90 days in milk) have a better reproductive performances, as showed by Yang L. et al 2009. Finally, in the F1 we founded a negative correlation between the kilograms of milk protein and the percentage of milk protein, this could be explained by the fact that our data are referred to the period where the cows have the lactation pic, so the high milk production could influence the protein percentage causing a dilution effect.

TABLE 2
CORRELATIONS BETWEEN PRODUCTIVE/REPRODUCTIVE PARAMETERS FOR THE EXAMINED BREEDS. ONLY SIGNIFICANT COEFFICIENTS ARE REPORTED. **- P<0-01.

Variables	Modenese	F1	F2	Holstein
Days open/SPP	0.72**	0.7**	-	-
%proteins/SPP	-0.51**	-	-	-
%protein/days open	-	-	-	-
Kg protein/SPP	-	-	-	-
Kg protein/days open	-	-	-	-
Kg protein/%protein	-	-0.22**	-	-

IV. CONCLUSION

This study has allowed to obtain a series of information about the physiological and metabolic profile of the Italian autochthonous cattle breed Bianca Val Padana / or Modenese. This autochthonous breed, bred for centuries in a large area of the Po Valley, demonstrates that it maintains peculiar and interesting physiological parameters due to his the poor genetic selection. His good metabolic trim (low level of ketone bodies) associated with the optimal days open period allow to reach the goal of one calf per year, that is difficult to obtain in a normal Holstein herd. The data processing of the Modenese and its crossbreed concerning the days open period, percentage of protein in milk and number of inseminations indicate that it is a very valid breed from the point of view of the reproduction and production.

The few heads of Holstein used for this study, showed a ketonemia higher than the Modenese and its crossbreed, herd in the same condition of the Modenese, whereas that the animals were bred in the same conditions.

The comparative study between the races in the same breeding conditions is a fundamental tool to understand the correct reproductive and productive physiology of dairy cattle. A better resilience against the NEB and his adverse effects of

Modenese cattle could be one of the phenomena underlying their better reproductive efficiency, as shown from the data in this study.

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