ABSTRACT

KEYWORDS: Energy balance, diagnosis, diet, reproductive indices

The PhD thesis titled ”RESEARCH REGARDING THE ENERGY BALANCE AND ITS INFLUENCE ON THE REPRODUCTIVE FUNCTION IN DAIRY COWS” has the character of originality combined assessment of energy status, its influence factors and the impact on reproductive indices in dairy cows.

The thesis comprises of 218 pages and is divided into two parts.

The bibliography spans of 47 pages, was systematized in four chapters presenting data in the literature regarding: symptoms topic, considerations on energy status, assessment of influence factors and how to determine the energy profile in dairy cows.

The second part, of personal contributions, are conducted on a number of 106 pages and contains seven chapters, which gives purpose and importance of the research, materials and methods, determining the energy balance, assessment of the impact of microclimate factors, nutrition and rumination on the energy status and their influence on reproductive indices in dairy cows.

Presented data are supported by 7 tables and 11 figures inserted in the reference study, 22 tables and 42 figures inserted in the personal contributions.The bibliography list totals 201titles of local and international literature, standards of working methods and files containing specialized internet. In the thesis are found own data published in scientific papers presented at the symposia with international participation organized by the Faculty of Veterinary Medicine Iassy.

The importance of thesis derives from thesis aimed at assessing the combined energy status, its influence factors and the impact on reproductive indices in dairy cows, this approach

provides the possibility of performing a three-dimensional implications for assesing energy status in animals and its influence on reproductive function in dairy cows.

Establish a health surveillance program metabolic allows early identification of metabolic disorders and their causes, namely the discovery of disturbance in feeding, or use of feed, and some toxic influences, especially on parenchymal organs.

Nutritional-metabolic surveillance is a complex diagnostic system that includes: morphoclinical examination of animal feed analysis and ration; examinations of energy profile and therapeutic test.

In the current socio-economic conditions, assessment of factors influencing energy balance is another imperative measure. These factors can be the basis to achieve an experimental model that can estimate energy balance orientation positively or negatively.

Based on these considerations, this paper has the following objectives and activities:

1. Perform additional tests to assess the energy balance:
   - Haematological examination in dairy cows;
   - Biochemistry tests;
   - Perform ultrasound;
   - Dosage of progesterone.

2. Assessment of factors influencing the energy balance:
   - Findings of differential feeding dairy cows;
   - Assessment of microclimate factors;
   - Findings of the process of rumination in dairy cows.

3. Correlations regarding the influence of the energy profile on breeding indices in dairy cows.

Chapter VII sought to determine the energy status of dairy cows after performing biochemicalexaminations, hematological and progesterone dosage.

In the present study, (for the determination of the energy profile by means of haematological and biochemical tests), 40% of the cows examined showed a normal sexual cycle, the remaining 60% had different sexual dysfunction cycle.

In the 40% of clinically healthy cows were formed two control groups: Advanced lactating cows (group M), and early lactation cows (M1 group). The 60% of cows with disorders of sexual cycle were divided into 3 groups: the first group comprises females with postpartum anestrus (G1)

was 20%, in the second group are included those with impaired ovarian cyclicity (G2) 20%, in the third group are cows with prolonged luteal phase (G3) 20%.

Delay resumption of postpartum ovarian cyclicity was the most prevalent condition, but did not affect the fertility of cows investigated. During the puerperium there is a distinct activity on ovarian follicle growth, present in most cows ovaries present in the cortical area of primary and secondary follicles, of which, through the selection and dominance process the dominant follicle will form about 11 days after parturition .

This growth and follicular development is significantly more pronounced in pregnant uterine horn contralateral ovary, suggesting an inhibitory action of gestation and / or CL of pregnancy on subsequent follicular development. Based on ultrasound and P4 dosages established that first postpartum ovulation in dairy cows with normal puerperium affected by ovarian cyst occurs between 15 and 21 days postpartum.

Anovulatory period is correlated with the appearance of the first dominant follicle and number of dominant follicles present before that ovulations. A prolonged interval elapsed between calving and first ovulation is due the ovulation was not produced, rather than lack of development dominant.

Cows that calved during winter (December-March) had a prolonged ovarian activity. Prolongation of the luteal phase occurred in 15.5% of cases examined postpartum. After calving, cows usually present a slight increase in dry matter intake, a fast increase in milk production and increased mobilization of fat tissue, which produce negative energy balance.

At 100 days of lactation, production from the four groups of cows was not affected, but the group G2 showed a low protein content in milk, leading to an increased ratio of fat and protein compared with normal group. Fulkerson et al. (2001) demonstrated that cows with a low content of protein in milk (2.89%) have a negative energy balance more severe and prolonged compared to cows that have a rate of 3.1% protein in milk.

Blood samples were collected every week before the feeding after milking between 7.30 and 9.00, in order to determine the protein, albumin, urea, unsaturated fatty acids, cholesterol, AST, ALT, glucose, calcium, phosphorus, magnesium, hemoglobin , serum protein, hematocrit and white blood cells.

The milk samples were taken twice daily at 07.00 and 17.00, and the production was recorded electronically. During the presence of colostrum or mastitis, milk was collected separately and weighed manually.

Determination of progesterone was performed to detect estrus and early pregnancy. These cattle were inseminated on day 0 of oestrus. Were collected 3 samples of milk, about 5 ml of each cow during the first 3 days after IA and then on days 10-12 şi 21-22 postinsemination. Progesterone analysis was performed using ELISA test. This cows were transrectal examined after 60 days from insemination for the diagnosis of pregnancy.

Cows with postpartum anestrus (group G1) following variations were found compared with healthy cows in group M: total serum protein (g / dl) in the lower level of the species, compared to cows in group M (7.2 ± 0.3 versus 8.79 ± 0.5) slightly increased serum albumin, serum urea (mg / dl) to the lower level of the species; alkaline reserve below the lower limit of the species in both groups of cows, serum enzymes, ALT above the upper limit of the species, and alkaline phosphatase, FA to the lower level of the species, indicating phospho-calcium metabolism disorders, mineral profile at the lower level of serum calcium values and the phosphoremy, a Ca / P ratio of 1.4.

Biochemical parameters in cows with prolonged luteal phase (group G3) showed the following changes compared to cows in group M1, healthy cows in early lactation: lower levels of total serum protein and serum globulin increases, elevated serum enzymes: ALT (U / l) above the upper limit of the species (45.50 ± 0.2) and low at the lower limit of the species in alkaline reserve, serum enzymes AST, GGT, AP, serum calcium and fosforemia indicating a state of metabolic acidosis and some liver disorders and phospho-calcium metabolism disorders.

Cows with ovarian cyclicity disorder (group G2) were found following average values compared to cows in the control group M: total serum protein (g / dl), lower value (6.3 ± 0.4 to 8.79 ± 0.5), serum albumin (%) increased to the upper limit of the species (53.55 ± 0.6 versus 41.0 ± 1.0), serum urea (mg / dl) with average values below the lower species (7.4 ± 0.9) serum enzymes: ALT above the upper level of the species (U / l), (63.4 ± 2.6), lower values of serum calcium and phosphoremy to control groups at the lower level of the species, indicating some liver disorders and phospho-calcium metabolism disorders.

Alkaline reserve (mEq / l) recorded low values, under the variation limit of the species in all the cows studied.

The values of the cows studied were included in the erythrocyte series physiological limits specified in the literature. Some constants of erythrocyte values were located towards the lower limit (hematocrit and erythrocytes).

VEM and HEM were located at the upper limit of the reference, the body drives to compensate anemia by releasing in blood RBCs (red blood cells) large.

Leukocytes showed variations in leukocyte fractions in experimental groups G1 and G2 compared to control group, values downward lymphocytes, monocytes and neutrophils increased, eosinophils and basophils.

Number of erythrocytes and hematocrit showed a decrease, the latter having an average of 34.2 ± 0.30 in the control group M1, compared to group M in advanced lactating (33.2 ± 0.83) and hemoglobin increased from 10.6 ± 0.20 to 14.1 ± 0.60.

Lymphocytes and monocytes showed a slight decrease, and neutrophils, eosinophils and basofills a slight increase. The results indicated some deficiencies in the energy profile in dairy cows, which causes hormonal disorders.

In the 94 cows examined on days 10-12 of the oestrus, progesterone level at 36.17%, 43.62 and 20.21 ± 2.14% was 0.08, 3.95 ± 0.46, 6.04 ± 0.21, respectively ng / ml. During the middle of the estrous cycle, progesterone values ranged from 1.12 - 8.31 ng / ml. On days 21-22 postinsemination examined 84 cows, these cows progesterone level being 6.85 ± 0.32 ng / ml to 16.67% cows, 4.08 ± 0.12 to 32.14%, 2.77 ± 0.05 ng / ml to 10.71% and 0.14 ± 0.03 ng / ml to 40.48% cows.

Determination of progesterone was performed to detect estrus and early pregnancy but milk progesterone measurement can be done in the following situations to:

- Detection of silent heats. Progesterone concentration indicates that the animal is in heat or approaches;
- Determination of estrus if: cow was observed in heat, but was previously diagnosed as pregnant, if the heats were observed, but the interval between estrus periods were abnormally long;
- Evaluate the accuracy of heat and discovering errors in detection of estrus;
- Detection of cows that have not calved;
- Monitoring postpartum ovarian status;
- Different types of ovarian cysts;

- Evaluation of response to different hormonal treatments.

Progesterone level is low on days 19 to 24 after birth, so that explains the females in oestrus detection rate of 95%.

It should be noted that other conditions in addition to the pregnancy, an increased level of progesterone causes in days 20 to 23 after birth. These causes are uterine infections, ovarian cysts, estrous cycle in days 24-28 and early embryonic mortality. Physiological amounts of progesterone in milk fat free or low fat, are between 0.00 - 12.60 ng / ml.

In research conducted during oestrus were able to observe clear and filant vaginal discharge and specific for this period to 23% of cows.

From the 100 cattle studied, 94% of them had the concentration of progesterone below 1 ng / ml from 0.01 - 0.90 ng / ml, and 6% of the cows in the study progesterone concentration was above 1 ng / ml, from 3.31 - 7.44 ng / ml. Progesterone levels above 1 ng / ml was considered during the luteal phase. Cows assumed to be in estrus were examined clinically and transrectal to confirm the results. Thus, 94% of the animals were in estrus and the remaining 6% of cows had higher values, because they have been inseminated in the luteal phase.

From thus 94% of dairy cows were confirmed to be in estrus on the basis of the progesterone level, 45.75% progesterone had an average of 0.04 ± 0.004 ng / ml on Day 0 of the estrus. The 42.55% of the animals, the concentration of progesterone was 0.016 ± 0.25 ng / ml, while 11.70% of the average was 0.66 ± 0.03 ng / ml.

In the 94 cows examined on days 10-12 of the cycle, the progesterone to 36.17%, 43.62% and 20.21 ± 0.08 was 2.14, 3.95 ± 0.46, 6.04 ± 0.21, respectively ng / ml. During the middle of the estrous cycle, progesterone values were 3.72 ± 0.16 ng / ml, so that the limits of variation ranged from 1.12 - 8.31 ng / ml. On days 21-22 postinsemination we examined 84 cows, these cows progesterone level was 6.85 ± 0.32 ng / ml to 16.67% cows, 4.08 ± 0.12 to 32.14%, 2.77 ± 0.05 ng / ml to 10.71% and 0.14 ± 0.03 ng / ml to 40.48% cows.

Progesterone milk, on days 20-21 postinsemination had an average of 2.69 ± 0.29 ng / ml, the limits of variation ranging from 0.01 - 8.57 ng / ml. Of the 84 cows examined on days 21-22 of which postinsemination at 50 of them, respectively 59.52%, the concentration of progesterone had more than 2 ng / ml, this results that were pregnant. The remainder of the 34 cows that had 40.48% progesterone levels below 1 ng / ml, the deemed non-pregnant. On day 31 after insemination was performed ultrasound exam to confirm the pregnancy diagnosis at those 50 cows.

In progesterone examination assay were found 50 pregnant cows, of which only 45 of them confirmed the diagnosis, in the other five it was assumed that embryonic mortality occurred.

Chapter VIII allowed to assess the microclimate factors that influence the energy balance.

Microclimate factors play a major role in directing the energy balance, the deficienciesencountered in the shelters can lead to increased energy consumption, which means increased energy needs provided by food.

Failure to provide necessary nutrients to maintain energy balance positivity leads to developmental disorders, decreased production, systemic disorder that can affect reproductive entire herd.

Assessment of microclimate factors was accomplished for a period of 30 days, both farm A and farm B, the observations are performed at the same time of the year, for both farm, so that the data can be compared, with different climatic influences .

Assessment of microclimate factors involved assessing body condition score at two lots of 100 dairy cattle each from the two farms, the lots with a similar distribution to confirm the total number.

Microclimate factors investigated were represented by temperature, humidity, airflow, noise and presence of noxious gases from shelters.

For farm A, from north east in Romania, the temperature data recorded during the 30 days had a mid value of + 22.34 ° C, within the range of + 12.2 ° C to + 29.8 ° C.

In farm B, the regional climatic conditions, temperatures recorded lower values by about 3° C on average, the lowest value recorded was + 6.3 ° C and maximum +24.3 ° C. Average temperatures for the 30 days of study on environmental conditions equaled + 17.8 ° C, about 5 ° C lower than the data in farm A. It considers values between + 10 and +20 ° C as the values that provides thermal comfort without additional consumption of energy, which would be obtained by increasing the nutritional value of feed rations.

The highest average value for relative humidity recorded in the shelter farm was 47.1% while the lowest recorded value of 43.8%. For farm B, the average daily values were increased, the maximum being 50.4% and 47.1% reaching minimum.

Inconsistency in the optimal values recorded in farm A (0.15 - 0.25 m / sec), though without exceeding the limits in terms of speed of air currents in shelters, correlated with respiratory

pathology seen in this farm, which affects energy balance of animals through increased energy consumption, also a decreased weight gain.

All values of microclimate factors were within the recommended limits in terms of farm B, while for the farm A the amount of air flow rate was increased to the optimum value without exceeding the limit, temperature values 20 ° C resulted in an increase of water intake and apathy cattle, resulting in the occurrence of the thermal stress associated with additional energy.

Chapter IX evaluated diet and rumination impact on the energy balance in dairy cows. Diet, is another factor that has an important role in maintaining optimal levels of reproductive function. Nutritional deficiencies are most often involved in the infertility conditions in dairy cows, especially those with high yields. Nutritional disorders cause metabolic disorders, immunological and hormonal changes that affects reproductive function and reproduction parameters in dairy cows. Infertility is caused by deficiency: quantitative and qualitative. Quantitative deficiencies are given by: overeating and malnutrition and qualitative are deficiencies in vitamin, minerals, trace elements, etc.

To meet both requirements on the production and maintenance of dairy cows in the farm A attempted providing nutrients needed by a different ratio of components forage rations. Thus, initially received a high amount of pasture (meadow) and then to be used an increased amount of barley, bran and alfalfa. The nutritional value of the two ratios were substantially equal, registering a higher value in initial ration for DM, UNL and UIDL and modified ration PDIN, PDIE, Ca and P, whichever is necessary to increase dietary intake of Ca and P due losses to milk production of these two compounds.

Both in the farm A and farm B, by changing feed rations reached an energy balance needed to satisfy the requirements for maintenance and production at the same time.

Also changes in the farm A brought in ration led to the installation of a positive energy balance, with implications in production and health of dairy cattle for the group under study.

Effects of rumination of the energy balance from the two farms were assessed by direct observation (farm A) or computerized record of rumination studied in cows (farm B). The evaluation was done by assessing its body condition score with a common development with energy balance.

The Hi-Tag program used in the farm B allowed assessment of rumination time, its association with the pathology observed in the studied cattle and milk production compared with data provided.

Rumination is a physiological process that allows the assessment of both the energy balance and reproductive disorders, the data obtained from this evaluation were correlated with the physiological status of the animal.

On farm A, rumination observation was made directly by farm staff, the data obtained is then registered in order to be interpreted later. Direct assessment of rumination process presents numerous obstacles, it is assessed subjectively during the day, making assessment of the overall, importance giving to animals at which the rumination has stoped.

The cattles with reproductive disorders and that abnormal body condition score represented 69.56% dairy cattle of the total, in which were observed rumination disorder, while the cows with liver disease and those with impaired phospho / calcium represented 26.08% and 4.34% of bovine rumination disorder.

Assessment of microclimate factors, nutrition and rumination gives relevant information on energy status of dairy cows, these factors having an overwhelming influence on the energy balance.

Chapter X allowed the estimation of correlations on the influence of energy balance on reproductive indices.

In order to assess reproductive indices were formed 3 groups of 30 dairy cows each. The control group (M) was formed from cows with positive energy balance, group I consisted of cows with negative energy balance due tomicroclimate deficiencies and group II consisted of cattle with negative energy balance subsequent bz increased production of milk, unsupported by a forage ration to allow compensation for losses. Setting lots watched to contain each an equal number of cattle (six dairy cows) in different lactations (I - II, II - III, III - IV, IV - V, V - VI), such assessment being the more accurate the whole herd.

The time elapsed between calving and the appeareance of the first sexual cycle is close to time elapsed between the occurrence of the sexual cycle and conception, which allows the formation of a model for assessing the total time for calving interval.

In lactation II – III, of lot I, the interval between two calvings averaged 402 days.

There is a severe damage to reproductive indices in group II, lactation III - IV, these changes are the consequence of increased milk production. Thus, there is an average of 113 days between the time of calving and conception, which increase the time interval between two calvings. Given the desire of farmers to obtain a product / animal / year, it is clear that at least for the category of dairy cows in group III, negative energy balance has a profound impact on reproductive function causing significant financial losses.

Regarding lactation IV – V, it is exceeding the optimum average of 365 days between two calvings both group I and II, negative energy balance leading to changing reproductive indices for the categories analyzed.

Following the decrease of milk production recorded in lactation V – VI, category most affected by negative energy balance evolution in terms of reproduction is the category in which there are microclimate deficiencies, which may lead to the development of secondary organopathies will mantain negativity energy status of animals.

Referring to the interval between calving and the appeareance of the first sexual cycle for lactations I - II and II - III there is a tendency to reduce the influence of negative balance on reproductive indices in the category of dairy cows with high production, as opposed to group I, which is recorded the most marked changes in reproductive parameters.

Increased average time between calving and conception delayed recovery may result due to negative energy balance and subsequent rise due to increase milk production.

The study allowed the observation of prolonging gestation for lactations IV and V in group I cows. Explanation could be established of a deficency fetal development as a consequence negative energy status of the mother.

Regarding the calving interval, it appears that the influence of negative energy balance on the reproductive index is reduced in the first two lactations for group II subsequently profound impact of negative energy status result in failure to achieve an economic objective product / animal / calendar year.

If the first gestations, the influence of severe negative energy balance is reflected on the total number of inseminations to conception, from the fourth pregnancy, the influence of negative energy status is not so striking compared to the group of dairy cows showing energy balance positive.