EFFECT OF GRAIN SOURCE TO GOAT MILK YIELD AND IMMUNOLOGICAL PARAMETERS

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Abstract. The milk of goats is mainly reserved for cheese making and therefore a quality evaluation of the milk is of fundamental importance. Goat milk composition is strongly influenced by goat nutrition, especially in highly productive animals. The feeding trial was carried out in organic goat farm for 135 days totally. The goal of our investigation was to assess the influence of different feedstuffs – wheat (wheat group –WG), oats (oat group – OG) and barley (barley group – BG) – on organic Alps goat milk quality indices. The highest milk yield was reached with the goats of the Oat group (2.88 kg per goat per day), which received feed ration with oat grain ($p \leq 0.05$). The lowest milk yield was shown by goats of the Wheat group (2.15 kg per goat per day), but the content of protein (2.84%) and milk fat (4.56%; $p \leq 0.05$) was highest in this group. The highest goat milk somatic cell count (SSC) was in barley group (301 μL^{-1}) and the lowest (213 μL^{-1}) - in wheat group ($p \leq 0.05$). The goat group, which in feed ration received barley grains, showed a reducing count of milk active T-, B-, D-cells and an increasing count of O-cells what is indicative of the changes in the immune system. A relatively high level of lymphocytes, active immunocompetent cells and monocytes (macrophages) and a low level of lysozyme and CIC are characteristic of a good immunity status of goats; in this case, it was provided the inclusion of wheat grain into the feed ration. After examining the various fodder additive effects on goat milk cytological and immunological parameters we came to a conclusion that wheat had greater advantages than oats whereas barley grains were given the last position.

Keywords: goat, milk, nutrition, cytological indices, immunological indices

Introduction. The production of goat milk and the popularity of goat milk products have been increasing steadily over the past few years. Over recent years, the tendency has been a slight reduction in the number of animals bred, while at the same time there has been a general increase in the volume of milk produced. The milk of sheep and goats is mainly reserved for cheese making and therefore a quality evaluation of the milk is of fundamental importance (Pirisi et.al., 2007).

Proper nutrition is essential for the health and productivity of all animals and is the basis of successful production systems. Goat milk composition is strongly influenced by goat nutrition, especially in highly productive animals.

Grain sources and processing have been discussed for many years. Feeding values differ among grain sources and processing methods. Cattle performance results indicate that metabolizable energy values for barley and oats are considerably higher than published tables imply and that metabolizable energy responses to steam flaking generally have been underestimated. Energy availability consistently was lower for milo than for other grains (Owens et. al., 1997).

According to the Sormunen–Cristian research, ruminants adapted more quickly to diets containing barley than to those containing oats. Compared to oats, the total daily DM intake was higher on barley. Hay consumption was significantly lower on oats than on barley. On oats the cattle experienced energy and protein deficiencies with their energy and protein intakes being 20% below feeding recommendations (Sormunen-Cristian , 2013).

Milk fat concentration is markedly affected by the net energy (NE) balance, dietary NDF content and dietary supplementation with oils. Milk protein content, and its characteristics, is more difficult to change than milk fat, although dietary energy seems to have a major role, while diet protein and amino acid supplementation only marginally affect milk protein level and its characteristics. Nutritional stress and some vitamins affect the somatic cell content of milk and this impacts cheese yield and quality (Pulina et al. 2006).

The presence of cells in bovine milk, the so called somatic cells, has been recognized and studied for many years. Goat milk contains on average a higher somatic cell count (SCC) than cow milk which is due to the apocrine character of milk secretion in goats, namely involving the destruction of the milk-producing cell and it reaching the light of milk-producing alveolus. High milk production was accompanied by low SCC, which might be associated to a dilution effect (Fernandez et.al, 2008).

Somatic Cell Count (SCC) is the total number of leukocyte cells per millilitre in milk (Miller et al., 1986). SCC in the milk is often used as an indirect measure of mammary infection status (Shook and Schultz, 1994; Caraviello et al., 2005). These cells originate from blood cells and play an important role in the metabolism of the mammary gland (Burvenich et al., 1994). The majority of the cells in somatic cell counts are leukocytes and others are cells from the udder secretory tissue (epithelial cells) (Bradley and Green, 2005). The epithelial cells are shed and renewed in the normal body processes. The white blood cells serve as a defence mechanism to fight disease infection and assist in repairing damaged tissue (Ma et al., 2000). The white blood cells are mainly composed of

Macrophage (MAC), Lymphocyte (LYM) and Polymorphonuclear Neutrophil leukocytes (PMN) (Dosogne et al., 2003; Bradley and Green, 2005). A lot of factors such as season, parity, lactation stage, nutrition, sanitation, environment, management and genetic factors could influence the level of SCC in bovine milk (Green et al., 2006, 2008; Heuven et al., 1988; Sheldrake et al., 1983). High levels of SCC in milk could result in great loss of milk yield and have negative effect on its quality, life time and processing traits (Barbano et al., 1991; Jones et al., 1984). SCC is mainly composed of leukocytes produced by the animal's immune system to fight an inflammation in the mammary gland or mastitis. SCC provides an indication of the healthy condition of mammary gland in an individual animal or in the herd if bulk milk is used and it is a hot topic in recent studies.

The HSCC (high SCC) resulted in higher pH values in milk and in higher moisture and lower fat contents in fresh cheese curds. Moreover, a lower recovery of fat and whey proteins was obtained from the HSCC than from the LSCC (low somatic cell count raw milk). The crude protein and casein contents were higher in the HSCC than in the LSCC curds during early and mid-lactation; an opposite trend was observed in late lactation (Albenzio et. al., 2004).

There are significant correlations between the level of milk somatic cell counts (SCC) and polymorphonuclear neutrophilic leukocytes (PMN); lower correlation coefficients were found between SCC and PMN for samples of bulk tank milk than for milk samples from individual animals. There is also a significant seasonal influence on milk PMN content and higher proportions of PMN are found in milk of animals calving in the spring than in milk from autumn calving cows (Kelly et. al., 2000).

Improvement of goat breeding, care and feeding in farms increases goat milk yields and highly productive goats reach more than 800 kg of milk in lactation. Such high rate of milk provides genetic potential, which has been achieved through targeted animal selection, selection and assessment, as well as improving goat feeding (Piliena and Spruzs., 2007; Spruzs, 1996).

Goats fed on high-quality and optimized feed ration provide the production of high quality milk and dairy products. Improper and poor feeding during lactation period reduce milk yield, weaken animals′organism, and has an influence on breeding and fertility ability and metabolic processes in goats. The objective of our investigation was to assess the influence of different feedstuffs – wheat (wheat group –WG), oats (oat group – OG) and barley (barley group – BG) – on organic Alps goat milk immunological parameters.

Material and methods. The feeding trial was carried out in Latvia farm "Livi", Madona District from May 15 to September 12, 2010, i.e. for 135 days totally.

In the preparatory period, which was lasting for two weeks, feeding, keeping and rearing conditions were equal for all 10 Alps goats included in the trial. Second and third lactation goats with a similar live weight (50–60 kg), body composition, and nutritional level were included in the group. During the accounting period, goats received feed produced in the farm: pasture grass and grains (Table 1). In the first 45 days of experiment goats received oat grain as additional feed, in the next 45 days oats were replaced by barley grain and then barley was replaced by wheat grain feed.

Table 1. **Trial scheme**

Group	Animals per group	Trial data	Feed ration
Oat group (OG)	10	15.May-28.June (45 days)	Pasture grass – 6.5 kg Oats grain – 0.8 kg
Barley group (BG)	10	29.June-12.August (45 days)	Pasture grass – 6.5 kg Barley grain – 0.8 kg
Wheat group (WG)	10	13.August-16.September (45 days)	Pasture grass – 6.5 kg Wheat grain – 0.8 kg

Table 2. **Chemical content of feed ration**

Group	Feedstuffs	Amount, kg	ME, $MJ\ kg^{-1}$	Digestible protein, g	Ca, g	P, g	Carotene, mg
OG	Pasture grass	6.5	15.93	124	19.5	4.55	260
	Oats grain	0.8	8.18	72	0.8	2.40	-
	Total:	x	24.11	196	20.3	6.95	260
BG	Pasture grass	6.5	15.93	124	19.5	4.55	260
	Barley grain	0.8	9.04	56	0.4	2.40	-
	Total:	x	24,97	180	19.9	6.95	260
WG	Pasture grass	6.5	15.93	124	19.5	4.55	260
	Wheat grain	0.8	9.08	64	0.3	3.20	1
	Total:	x	25.01	188	19.8	7.75	261
		Requirement:	23.3	180	13.9	9.9	50

Nutrient requirement in goats was determined according to animal live weight and milk yield following the normative regulations adopted in Latvia and the United States (Spruzs J., 2005; Nutrient Requirements..., 1981). During the trial, all goats were in mid-lactation stage – 3–8 months post parturition.

According to the catalogues of feedstuffs (Ositis U., et al 2000; Latvietis J., 1996), feed rations for the Alps goats of all groups were practically of equal value by the amount of metabolic energy (ME), crude protein, digestible protein, calcium (Ca), phosphorus (P) and main biologically active substances (Table 2).

During the trial, the goats received mineral supplement KNZ – 100 containing 99% of NaCl.

During the trial, milk yield produced by each goat was measured with a precision to ±0.05 kg. Milk fat, protein, and lactose content was determined by a daily average sample once a month using *Milko Scan 133* according to the IDF standard 141C:2000 requirements and somatic cell count in accordance with standard LVS EN ISO 13366-3:1997 requirements.

Haematological study and cytological analysis of milk were carried out using a microscope. To assess the immune status of goats the following parameters of milk were investigated: T-and B-cell count (Гришина et al., 1978); lysozyme level (Грант et al., 1973); circulating immune complex (CIC) content (Riha et al., 1979). A specific preparation of milk samples was used. To analyze cell composition milk was washed by Eagle solution, centrifuged and re-suspended. The humoral immunity of the organism was characterized using natural resistance indices – the amount of lysozyme and circulating immune complex (CIC) in milk. For analysis, milk samples of 40 ml were centrifuged at 3000 rev/min for 25 minutes. Lysozyme was determined by spectrometric method (Грант et. al., 1973). The CIC level was determined spectrophotometrically by precipitation reaction with polyethylenglucole (Riha J., 1979). CIC is the antigen-antibody compound, and in case of the inflammatory process the concentration of CIC increases.

The results were statistically processed using Microsoft Excel, Student t – test ($p \leq 0.05$).

Results and discussions

During the trial, the highest milk yield – 1296.0 kg or 2.88 kg per goat per day ($p \leq 0.05$) or by 33.9 % more compared to WG – was reached with the goats of the OG, which received feed ration with oat grain (Table 3).

Table 3. **Alps goat milk yield during the trial, kg**

Group / days	Milk yield per group	Milk yield per goat per 45 days	Milk yield per goat per day
OG / 45 days	1296.0	129.6	2.88*
BG / 45 days	1008.0	100.8	2.24
WG / 45 days	967.5	96.8	2.15
*p≤ 0.05			

The lowest milk yield was shown by goats of the WG – 967.5 kg or 2.15 kg per goat per day, which received wheat grains as concentrates. The decrease of milk yield in this case can be explained by the stage of lactation (second part of lactation).

The highest protein content (2.84 %) in goat milk was when Alps goats received a daily ration with wheat grain. Also the highest milk fat content (4.56 %; $p \leq 0.05$) was from goats, which received pasture grass with wheat grain (Table 4).

Table 4. **Chemical composition of goat milk, %**

Group	Milk fat	Milk protein
OG	3.66 ± 0.20	2.80 ± 0.03
BG	3.88 ± 0.11	2.69 ± 0.03
WG	4.56 ± 0.10*	2.84 ± 0.03
*p≤ 0.05		

Consumption of metabolic energy and digestible protein necessary for 1 kg goat milk production is presented in Table 5.

Table 5. **Consumption of ME and digestible protein for 1 kg milk production**

Group	ME, MJ kg^{-1}	% to OG	Digestible protein, g	% to OG
OG	8.37	100.0	68.1	100.0
BG	11.15	133.2	80.4	118.2
WG	11.63	138.9	87.4	128.3

Table 6. **Goat milk SCC in 1 μL**

Group	Total	Segment-nucleus	Lympho-cytes	Mono-cytes
OG	244±22*	194±20*	36±4	4±1
BG	302±40*	253±43*	43±7*	4±2
WG	213±5*	174±8*	39±1	6±2*
* p≤ 0.05				

Table 7. **Immunological indices of goat milk, μL**

Group	T-cells	B–cells	D-cells	O-cells
OG	8.0±1.0	4.0±0.1	3.0±0.2*	24.0±2.0*
BG	6.0±0.6*	2.2±0.4*	1.4±0.5	31.6±5.7*
WG	15.0±2.0*	7.0±0.2*	5.0±1.0*	21.0±1.0*
*p≤0.05				

Table 8. **Goat milk humoral immunity indices**

Group	Lysozyme, μg 100 ml^{-1}	CIC, ekstr.unitsx100
OG	31.90±6.71	10.30±2.99*
BG	38.78±4.94*	14.62±2.04*
WG	27.75±6.77	3.31±0.64*
*p≤0.05		

The best results were achieved with the goats of the oats group. These goats received oat grain as feed additive using only 8.37 MJ ME and 68.1 grams of digestible protein for 1 kg milk production.

It should be noted that the goats receiving the feed additive wheat and barley grain also achieved good results in consumption of ME and digestible protein for producing of 1 kg of milk.

The amount of goat milk somatic cells in 1 μL is shown in Table 6.

According Bernacha (2007), an average content of cell elements in the milk of the goats is 600 thousand cell elements 1 mL^{-1} in average, ranging from 10 thousand to 10,000 thousand, depending on the lactation month, which demonstrates that the hygiene quality of the examined milk is good. However, the maximum values of the somatic cell count are distressing, and thus the goats in which the highest content of cell elements in milk kept repeating should be culled from breeding in the future in order not to deteriorate the quality of the raw material obtained. Bernacha found that increase in the somatic cell count in milk was accompanied by an increase in the daily milk yield as well as its content of fat, but we did not find the same coherence in our investigation with Alps goats. The white blood cells are mainly composed of Macrophage (MAC), Lymphocyte (LYM) and Polymorphonuclear Neutrophil leukocytes (PMN) (Bradley and Green, 2005). The highest goat milk SSC was in barley group (302 thous. mL^{-1}; $p \leq 0.05$) and the lowest in wheat group (213 thous mL^{-1}; $p \leq 0.05$), which indicates a good hygienic quality of milk and good health condition of goat's udder. The total increase in the goat milk SCC as well as increase in the content of segmentnucleus cell can be seen as a negative tendency in barley group. This points to the possibility of inflammation and could be associated not only with feed but also with a very hot summer time, which was during the feeding experiment. Hot weather reduces immunity and opens the way to illness. Paape and Capuco (1997) claimed that neutrophils made up 50–70% of the somatic cell count in milk from goats free of intramammary infection whereas neutrophils only made up 5–20 % of the total cell count in bovine milk. During inflammation, the major increase in SCC is due to the influx of segmentnucleus (neutrophils) into the milk to fight infection and have been estimated at over 90 % (Harmon, 1994). In our case, segmentnucleus made up 80–83 % of the somatic cell count.

The absolute count of lymphocytes and monocytes was not statistically different in oats and wheat groups. The proportion of lymphocytes in goat milk may be as high as 10% or even 17% (Boutinaud et al., 2002; Bergonier et al., 2003). In the present study, we found that the percentage of lymphocytes reached 14–18%. It was shown in Schmaltz at al. (1996) study on dairy cows that the number of these subpopulations may increase in mastitis. The earlier study on dairy goats by Bergonier et al. (2003) showed that the percentage of lymphocytes did not depend on the presence of bacteria and reached only 2.5% whereas the percentage of monocytes in goat milk ranged between 10–15%, depending on the health status of the mammary gland (Bergonier et al., 2003). In the present study, the percentage of monocytes in goat milk ranged between 1.3–2.8%.

Lymphocytes are the only cells of the immune system that recognize a variety of antigenic structures through membrane receptors, which define their specificity, diversity and memory characters (Boyso et al, 2007). T-lymphocytes and B-lymphocytes are two subsets of lymphocytes that differ in function and protein products and play specific immune functions (Harmon, 2001). According to immunological indices (Table 7), the lowest absolute amount of T-and B-cells was found in the milk of barley group (resp. 6.0 $\mu L^{-1}$and 2.2 μL^{-1}) and but highest amount in wheat group (resp. 15.0 $\mu L^{-1}$and 7.0 μL^{-1}; $p \leq 0.05$).

B-lymphocytes are bursa-dependent lymphocytes; the precursors of antibody-producing cells and the cells primarily responsible for humoral immunity. T-lymphocytes are thymus-dependent lymphocytes; those that pass through or are influenced by the thymus before migrating to tissues; they are responsible for cell-mediated immunity and delayed hypersensitivity. T-and B-cells are the main immune cells, which provide the specific immune response. D-cells are lymphocytes with the highest activity because they have more cell membrane receptors, which are the first to be activated in immune response. 0-cells are lymphocytes that do not respond to T-, B-cell receptors, indicating a low activity of lymphocyte. If in the milk are less inactive lymphocytes (O-cells) compared with active lymphocytes (T- and B-cells), the goat immune defence is higher.

The amount of D-cells in the barley group (1.4 μL^{-1}) milk was lower compared to oat (3.0 μL^{-1}) and wheat (5.0 μL^{-1}) groups milk ($p \leq 0.05$). The largest O-cell absolute count was in barley group (31.6 μL^{-1}; $p \leq 0.05$) and the lowest in wheat group (21.0 μL^{-1}; $p \leq 0.05$), indicating the high immune cell functional state

Goat group, which in feed ration received barley grains, presents a reducing of milk active T-, B-, D-cells count and an increasing of O-cells count what is possibly indicative of the changes in the immune system. A relatively high level of lymphocytes, active immunocompetent cells and monocytes (macrophages) and a low level of lysozyme and CIC are characteristic of a good goat immune status, and in this case it was provided by including wheat into the feed ration.

The lysozyme content in goat milk (Table 8) was higher in the barley group.

The differences of lysozyme ratio in the oat and wheat groups were not statistically significant (Table 8). Lysozyme is a non-specific humoral immunity integral indicator, and it was significantly higher in the barley milk (38.78 μg 100 mL^{-1}) compared to the amount of lysozyme observed in oat and wheat groups milk ($p \leq 0.05$). Ruminants are comparatively lacking in lysozyme in tears, saliva, and milk (Prieur, 1986). Bovine milk contains 0.13 μg of lysozyme mL^{-1}, whereas goats and sheep produce 0.25 and 0.10 μg mL^{-1} respectively (Chandan et al., 1968). Accordong to our results, the amount of lysozyme is higher in goat milk. Because lysozyme limits the growth of some bacteria that cause intestinal infections and diarrhoea and also encourages the growth of other beneficial intestinal bacteria, it is considered to be one of the main components of milk that contribute to the health and well-being of milk consumers.

The level of CIC in wheat group goat milk was lower in comparison with the oats group and barley group ($p \leq 0.05$).

In goat group, which additionally received barley grain, milk lysozyme content is linked to increases of segmentnucleus cells content, as these cells produce lysozyme. BG goat milk CIC level was higher because of neutralization of exogenous antigen by antibody and formation of circulated immune complexes. Summarizing it can be stated that the barley trial group compared to oat and wheat groups of goats received worse fodder which together with the effects of bad weather worsened the immunological status of goats.

Conclusions

The highest milk yield – 2.88 kg per goat per day or by 33.9 % more compared to wheat group –was reached with the goats of the oat group, which received feed ration with oat grain. The lowest milk yield was shown by the goats of the wheat group – 2.15 kg per goat per day, but it had the highest protein content (2.84 %) and the highest milk fat content (4.56 %). The decrease in milk yield and the increase of milk fat and protein content in this case can be accounted for by the change of lactation stage (second part of lactation).The highest goat milk SCC was in barley group (302 μL^{-1}) and the lowest (213 μL^{-1}) in wheat group ($p \leq 0.05$) what indicates a good hygienic quality of milk and good health condition of goats' udders. The total increase in the goat milk SCC as well as increase in the content of segmentnucleus cells can be seen as a negative tendency in barley group. This points to the possibility of inflammation and could be associated not only with feed but also with a very hot summer time. During the inflammation, the major increase in SCC is due to the influx of segmentnucleus into the milk to fight infection and have been estimated to 80–83%. The absolute count of lymphocytes and monocytes was not statistically different in oats and wheat groups and the proportion of lymphocytes in goat milk reached 14–18%. Goat group, which in feed ration received barley grains, presented a reducing of milk active T-, B-, D-cells count and increasing of O-cells count and this could be indicative of the changes in the immune system. If in the milk are less inactive lymphocytes (O-cells) compared with active lymphocytes (T- and B-cells), the goat immune defence is higher, and it was seen in wheat group, where the total absolute amount of T- and B-cells was 22.0 μL^{-1}, but amount of O-cells was 21.0 μL^{-1}. Relatively high levels of lymphocytes, active immunocompetent cells and monocytes (macrophages) and a low level of lysozyme and CIC are characteristic of a good goat immune status, and in this case it was provided by including wheat grain into the feed ration. After examining the various fodder additive effects on goat milk cytological and immunological parameters we came to a conclusion that wheat had greater advantages than oats whereas barley grains were given the last position.

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