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Original Contribution

HISTOPATHOLOGICAL ALTERATIONS IN THE LIVER OF COWS, FED DIETS SUPPLEMENTED WITH COTTONSEED, SUNFLOWER SEEDS AND SOYBEAN

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ABSTRACT

The effect of supplementation of rations of dairy cows with cottonseed, sunflower seed and soybean on the liver was studied in 12 cows from the Bulgarian Brown Breed. The cows were divided into 4 groups with 3 cows per group. The basic ration was equal fro all groups and consisted of 20 kg maize silage, 10 kg pea-barley haylage and 2 kg alfalfa hay. Additionally, the cows of each groups received dietary supplements as followed: group 1 (control) – 3 kg grounded corn and 3..6 kg sunflower meal daily; group 2 – 3.5 kg cottonseed and 2.5 kg sunflower meal, group 3 – 2,3 kg sunflower seed, 3.0 kg sunflower meal and 0,8 kg grounded corn, group 4 – 4 kg soybean and 1 kg grounded corn. Study specimens were obtained by liver biopsy, done after the first and the second month of feeding the experimental diets. In addition, cows from group 2 provided study samples after five months of feeding and 2 months after feeding cessation. Cows given cottonseed for one month exhibited granular dystrophy and initial fatty infiltration of the liver, after a 2-month feeding. After 5 months, a high degree of fatty liver dystrophy was present. The feeding on sunflower seed did not result in any significant changes. The feeding on soybean resulted in moderate liver granular dystrophy.

Key words: dairy cows, cottonseed, sunflower seed, liver

INTRODUCTION

If the level of dietary energy intake in the beginning of lactation could be increased, it would result in high milk production during the entire lactation period (1) The inclusion of fats increases the energy content of the diet because of the calories they provide and the more efficient metabolism compared to volatile fatty acids that are formed in the fore stomachs (2). Fats comprise a relatively small part of dry matter of cattle rations (1.5-3%) but play a significant role in nutrition, so their amount is regulated in a number of countries (Denmark, USA). Apart being a source of energy, dietary fats play also a specific role as source of essential fatty acids and precursors for synthesis of milk fat (3).

A number of authors have observed a favourable effect of cottonseed and other feeds, rich in fats, on the milk productivity and the composition of milk (4, 5, 6, 7, 8, 9). The information about the effect of large amounts of dietary fats on the status of metabolically important organs, such as the liver, is however scarce. On the other hand, it is known that gossypol from the cottonseed, could result in intoxications in animals fed continuously with this product (10, 11).

The purpose of the present study was to investigate the effect on the liver, the time-related additions of relatively large amounts of cottonseed, sunflower seed and soybean seed, to the food rations of dairy cows.

MATERIALS AND METHODS

Experimental design and animals: The experiment was designed using the factorial method to test the effect of three sources of fats and protein in the ration of dairy cows. Twelve cows from the Bulgarian Brown Cattle breed, weighing 548-557 kg, were used. The cows were divided in 4 groups with 3 animals in each group. All cows were in the beginning of the lactation period (18 to 29 days after calving) and at the beginning of experiments had a daily milk yield of 24.5 to 25.8 kg. Cows were reared in stalls with automatic watering devices.

The basic ration was equal for all

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groups and consisted of 20 kg maize silage, 10 kg pea-barley haylage and 2 kg alfalfa hay. Additionally, the cows of each group received various concentrate feed twice daily – morning and evening. Group 1 (**control**) received 3 kg ground corn and 3.6 kg sunflower meal daily; group 2 – 3.5 kg cottonseed and 2.5 kg sunflower meal, group 3 – 2,3 kg sunflower seed, 3.0 kg sunflower meal and 0,8 kg ground corn, group 4 – 4 kg soybean and 1 kg ground corn. The four tested rations contained almost equal daily amounts of food units (19 to 19,1), crude protein (2944 to 2976 g) and crude fats (1028 to 1085 h).

Table 1 presents the chemical composition of used feeds, determined by the Veende method following the description in AOAC (1996)(12).

Table 1. Composition of feedstuffs

Feedstuffs	***DM,* g.kg^{-1}***	***Dry matter composition, g.kg^{-1}***			
		CP	*EE*	*CF*	*Ash*
Maize silage	314.8	89.5	31.3	223.5	59.3
Pea-barley haylage	495.9	125.2	36.5	270.0	87.3
Alfalfa hay	860.8	185.4	26.3	298.4	93.5
Cotton seeds	887.0	214.2	213.9	261.0	40.1
Sunflower seeds	876.8	194.0	340.3	251.5	38.7
Soybean seeds	880.4	390.0	201.0	70.8	52.9
Maize, grounded	867.6	105.7	39.7	25.6	18.0
Sunflower meal	887.9	366.4	19.9	278.0	74.9

** Abbreviations: DM – dry matter, CP – crude protein, EE – ether extract, CF – crude fiber*

Material for investigation from the liver was obtained by biopsy, using the routine method according to Angelov et al.(13). Specimens were obtained twice after the first and the second month of feeding the experimental diets. In addition, cows of group 2 provided samples after feeding for 5 months and 2 months after cessation of cottonseed supplementation, i.e. 7 months from the beginning of the trial.

The liver specimens were investigated histologically and histochemically. Part of them were embedded in paraffin and cut on a microtome, and another part - cut on a freezing microtome. The cross-sections were stained with:

- haematoxylin/eosin (H/E)– for general observation;
- Sudan III and according to Kay and. Whitehead for fats(14)
- according to Best for glycogen(14)
- according to Perls, modification of Banting for iron (14,15,16,17,18).

The investigations were performed in the Department of Pathoanatomy, Faculty of Veterinary Medicine, Stara Zagora and the Laboratory of Pathomorphology at the Central Research Institute of Veterinary Medicine, Sofia, Bulgaria.

RESULTS AND DISCUSSION

All examinations of livers of cows from group 1 (**control**) showed that their structures were normal. Hepatic lobes were entirely preserved. Hepatic plates and central lobular veins were normal, without alterations. At some sites, single hepatocytes, no more than 3-5%, were with inflated or lytic nuclei. Cells with double nuclei were also visible. In capillary networks of some lobules, there were single lymphocytes and neutrophil granulocytes. The reactions for fats, glycogen or iron were negative. There was no connective tissue growth, nor cirrhosis.

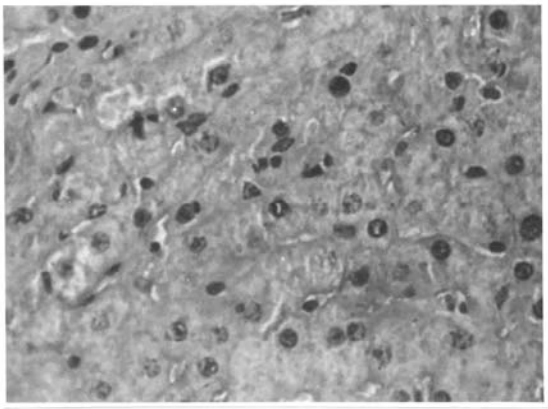

Figure 1. *Bovine liver after a 2-month feeding with the control ration, Magnification 400 x, H/E*

The most important histopathological and histochemical changes were observed in the cows from group II, whose ration was supplemented with cottonseed. The first examination by the end of the first month on the experimental diet showed moderate granular dystrophy in all three cows, affecting 50-60% of lobular hepatic cells. The changes were moderate and manifested by clearing of the cytoplasm and appearance of fine granular protein formations within. The cells' nuclei were not altered, but some of them, about 15-18%, were inflated, lytic or pyknotic (**Figure 2**). The reaction for glycogen was highly positive (+2 to +3).

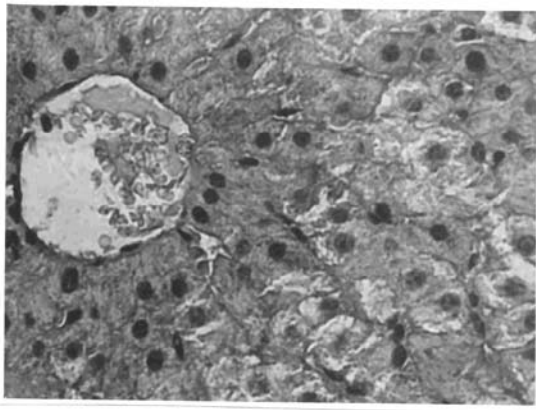

Figure 2. *Bovine liver after a 1-month feeding with cottonseed – moderate granular dystrophy Magnification 400 x, H/E*

The reaction for fats was weak. In some liver cells, whose nuclei were not damaged, the staining reaction for fats resulted in small yellow-reddish droplets or indistinct staining, indicative of fatty infiltration (**Figure 3**). The iron staining was negative. There were neither circulatory disorders, nor connective tissue growth.

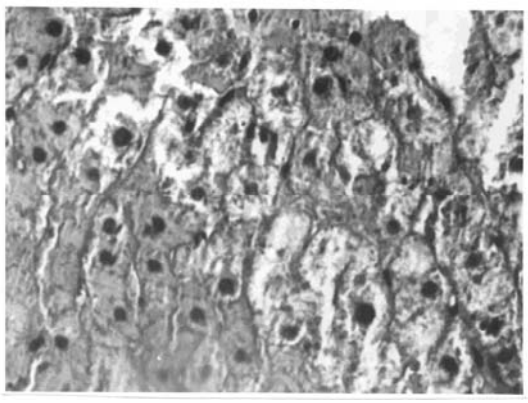

Figure 3. *Liver from cow No 5013 after a 1-month feeding with cottonseed – moderate fatty infiltration and granular dystrophy in an area with maximum alterations. Magnification 400 x, H/E*

During the second examination of the same cows after another month of supplementation with cottonseed, the granular dystrophy was fairly detectable, but instead, there was a fatty infiltration and fatty dystrophy, affecting 50-65% of cells in lobules (**Figure 4**). In one cow (No 3105), up to 95% of cells were affected (**Figure 5**). The staining of glycogen was still high: 2+ to 3+, indicating glycogen storage disorder, i.e. impaired carbohydrate metabolism. There was no connective tissue growth or circulatory troubles. The staining for iron was weak or negative.

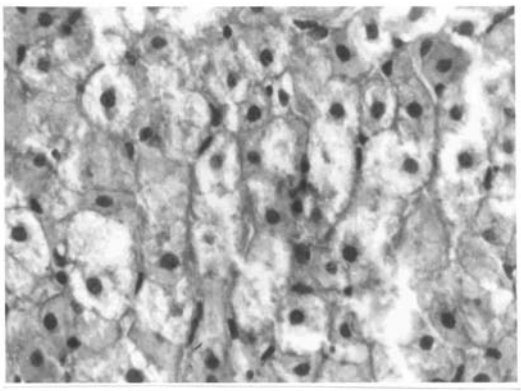

Figure 4. *Liver from cow No 5013 after a 2-month feeding with cottonseed – combination of fatty infiltration and fatty dystrophy of hepatocytes. Magnification 400 x, H/E*

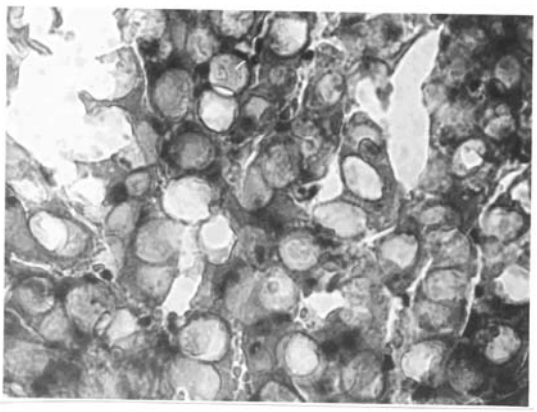

Figure 5. *Liver from cow No 3105 after a 2-month feeding with cottonseed – severe diffuse fatty dystrophy. Magnification 400 x, H/E*

The specimens from cows, fed on cottonseed for more than 5 months, showed a severe diffuse fatty dystrophy of the liver (**Figure 6**). The staining reaction for iron was slightly positive, and for glycogen – highly positive (3+). There were no circulatory troubles, nor connective tissue growth. These morphological data indicated a simultaneous significantly impaired fat and carbohydrate metabolism following continuous supplementation of the feed with cottonseed.

The cows from group II, given

cottonseed with the ration for 5 months, followed by a 2-month pause, exhibited a reduction of liver fatty dystrophy that ranged between 35-60%; in one cow the fatty dystrophy percentage was 35%, in another - 50% and in the third -60%. The size of fatty droplets in hepatocytic cytoplasm decreased and disappeared in the lobules' periphery, but they persisted large and numerous around the central veins (**Figure 7**). This is major evidence that the fatty dystrophy of the liver in cows, fed cottonseed, was reversible.

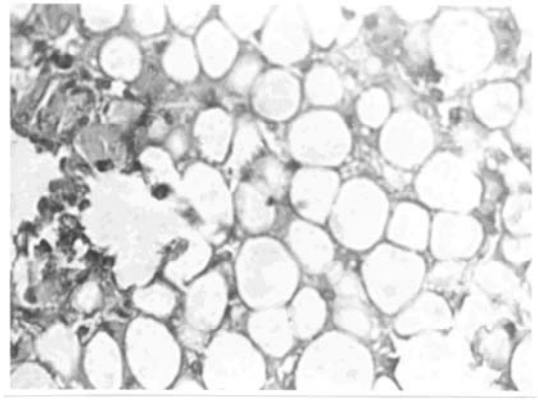

Figure 6. *Liver from a cow after more than 5-month feeding with cottonseed – severe diffuse fatty dystrophy. Magnification 400 x, H/E*

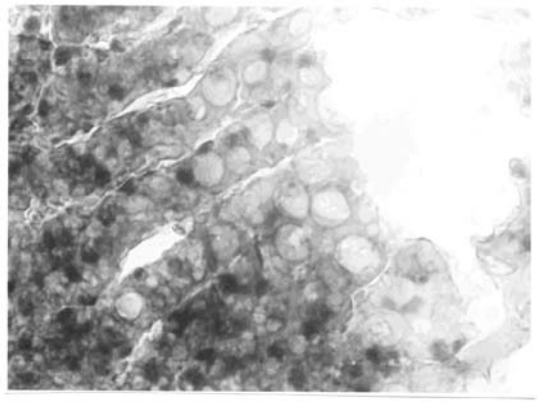

Figure 7. *Liver from cow No 3282 after being fed for 5 months with cottonseed, but after a 2-month pause –fatty dystrophy around the central veins of the lobule. Magnification 400 x, H/E*

In these cows, the staining reaction for iron was negative or slightly positive (+1) whereas for glycogen – moderately positive (1+ to 2+). There was also a granular dystrophy of about 15-16% of the tissue. No connective tissue growth was visible.

The cows supplemented with sunflower seed (group III) for 1 or 2 months, showed similar morphological findings. The liver biopsy exhibited slight granular dystrophy in 15-20% of cells (**Figure 8**) and often, the alterations were of the type of cloudy swelling (preceding the granular dystrophy). The staining reactions for iron, glycogen and fats were negative. There were no circulatory disorders, nor connective tissue growth.

The supplementation with sunflower seed is suitable because it does not result in considerable histological liver changes, as well as in any metabolic alterations.

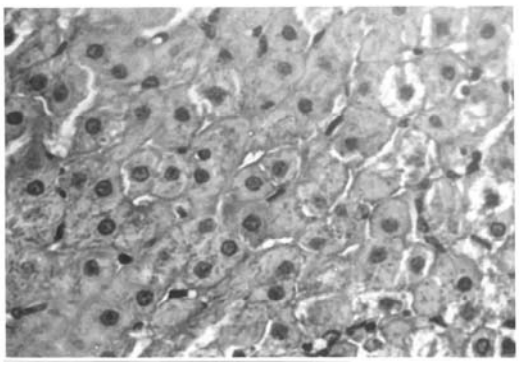

Figure 8. *Liver from cow No 7510 after 2-month feeding with sunflower seeds – single cells with granular dystrophy, almost normal. Magnification 400 x, H/E*

In cows given soybean (group IV) the first examination by the end of the 1st month showed a fine granular dystrophy of the liver over 15-20% and initial fatty infiltration of about 10-15% of cells (**Figure 9**). There was also moderate glycogen storage dystrophy ranging from traces to 2+. There were no circulatory troubles, no connective tissue growth; the staining for iron was negative.

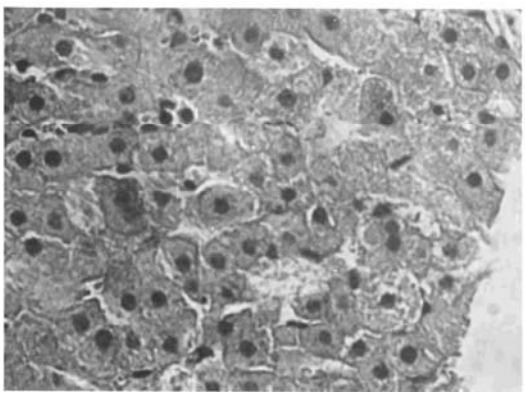

Figure 9. *Liver from cow No 6749 after 1-month feeding with soybean seeds – slight granular dystrophy. Magnification 400 x, H/E*

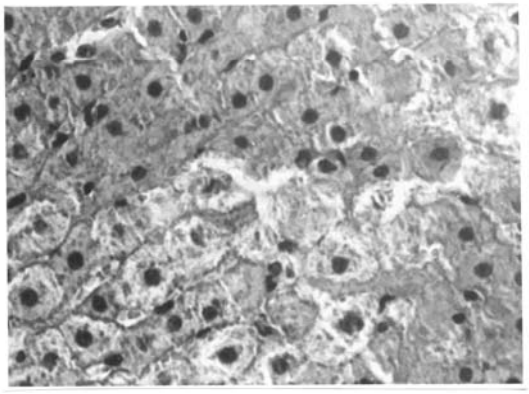

Figure 10. *Liver from cow No 7025 after 2-month feeding with soybean seeds –granular dystrophy and fatty infiltration in an area of maximum alterations. Magnification 400 x, H/E*

The second examination of the same cows after a 2-month feeding on the soybean diet revealed enhancement of the granular dystrophy and fatty liver infiltration (up to 20% for the former and about 25-28% of cells for the latter) (**Figure 10**). The glycogen reaction was up to 2+, the staining for iron – positive in one cow and negative in two. Neither fatty dystrophy nor cirrhosis was noticed.

CONCLUSION

The histological and histochemical investigations on liver biopsy specimens from cows, whose rations were supplemented for a long time with cottonseed, whole sunflower seed and soybean showed that more considerable morphological alterations were observed in cows given cottonseed, whereas the least ones were in those given soybean. The addition of sunflower seed to the ration resulted in insignificant changes, close to normal.

After a 1-month supplementation of cows' ration with cottonseed, there was a granular dystrophy and initial fatty infiltration, whereas after 2 months – diffuse fatty and glycogen dystrophy. The metabolism of fats, carbohydrates and proteins was progressively impaired. The continuous supplementation of the diet with cottonseed for 5 months resulted in severe diffuse fatty and glycogen dystrophy of the liver. After interruption of the supplementation for 2 months, the morphological changes decreased with clear tendencies towards normalization, i.e. the observed dystrophy was reversible and ended with normalization of the metabolism. A number of studies on the toxicological and pathological changes in calves (19, 20) demonstrated histological lesions of livers and numerous centrilobular necroses.

The supplementation of cows' ration with sunflower did not cause any considerable morphological and metabolic changes.

The feeding on soybean resulted in moderate granular dystrophy of the liver, with minimum impairment of fat and carbohydrate metabolism.

In all three feed supplements – cottonseed, sunflower seed and soybean, there were no hepatic circulatory changes, inflammations, necroses, and connective tissue growths.

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