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OVINE KETOSIS. VI. THE EFFECT OF STARVATION ON THE BLOOD LEVELS OF KETONES AND GLUCOSE IN PREGNANT EWES FROM THE KAROO AND THE HIGHVELD REGIONS OF SOUTH AFRICA

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It is generally recognised that hypoglycaemic ketosis of pregnant sheep is due to the combined operation of several factors, of which two, namely advanced pregnancy and sudden reduction of food intake, are essential. The number of animals which develop the disorder when only these two factors co-operate, has been shown to vary greatly. The incidence of the disease can, however, be increased by subjecting the animals to an additional stress factor such as either the onset of exceptionally cold weather (Dimmock, Healey & Bullard, 1928; Reid, 1960 a), or mechanical transportation (Reid, 1958). Previous work has shown that any of these factors acting independently has little or no effect on the levels of blood ketones and glucose in Merino sheep. Adequately fed monoctocous ewes in an advanced state of pregnancy did not show increased blood ketone levels (Procos, 1962 a). Starvation (Procos, 1962 b) and temperature drop (Procos, 1961) were independently capable of inducing, at the most, a mild ketonaemia and hypoglycaemia. Evidence which indicates the participation of yet further factors in the production of pregnancy ketosis in South Africa is presented in this paper.

The present paper reports a study of the blood levels of ketones and glucose during fasting of two groups of young preparturient ewes from different regions of South Africa. One group was reared on the Karoo where pregnancy ketosis of sheep (Domsiekte) is endemic and constitutes an economic problem (Van Rensburg, 1931; Henning, 1932), while the other group was reared on the Highveld where this metabolic disorder seldom occurs. The two groups arrived at Onderstepoort in consecutive years (1962, 1963) in early September for experimentation in early April of the following year. The ewes from the Karoo came from an area which had been drought-stricken for two years (De Wet, private communication) and were consequently in very much poorer condition on arrival than those from the Highveld; nevertheless, owing to subsequent adequate feeding, both groups of animals appeared to be in good condition when the experiments started. Two prefasting diets, one containing fresh-cut green lucerne and the other lucerne hay, were used, because Procos (1962 b) found that sheep fed the latter diet tended to have higher blood glucose levels than those fed the former one. The seasonal temperature drops to which the animals were exposed during experimentation were similar to those previously reported (Procos, 1961).

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Methods and Materials

Animal management

Two groups of 30 Merino ewes aged from 2·5 to 3 years were used. The Karoo group came from Laingsburg, Cape Province, while the Highveld group came from Makwassi, Transvaal. For about four months after their arrival at Onderstepoort, the ewes were kept in an open camp where they had free access to a stable and water, and were offered a diet of lucerne hay (*ad lib.*), cowmeal (maize starch 70, protein 17, vitamin-containing materials 13 per cent w/w) (200 gm approx.), Phosvita (mineral/trace-element lick, Ruffle) (*ad lib.*), and green fodder (thrice weekly). Twenty-eight animals from the Karoo and 22 from the Highveld were served by two rams over periods of 30 and 35 days respectively. The ewes for experimentation were selected for the closeness of their conception dates: 12 (within three days) from the Karoo group; and 11 (within two days) from the Highveld group, while the twelfth ewe (34) of this group conceived 35 days later than her group mates. These selected animals were subjected to laparotomy at approximately 10 weeks of pregnancy to verify that they were indeed pregnant. After the operation the animals were weighed at about weekly intervals and kept individually in small covered pens. The diets fed in the pens were similar in respect of cow meal and Phosvita to that fed previously in the camps, but contained either lucerne hay (1000 gm) or fresh-cut green lucerne (3000 gm) in addition. The two major groups of sheep were divided into two sub-groups, one of which received the lucerne hay diet while the other was given the green lucerne diet. The composition of these two diets in respect of crude protein and fibre fed to the Karoo ewes in 1963, and to the Highveld ewes in 1964 was as follows:—

Component	Green lucerne diet (gm day)		Lucerne hay diet (gm day)	
	1963	1964	1963	1964
Fibre	198·0	190·7	300·6	244·9
Crude Protein	160·5	147·4	172·1	172·7

All the food was invariably eaten. Fasting of the ewes began on the 135th day of gestation, except in the case of ewe 34 which was only 100 days pregnant, and lasted eight days. On completion of the fast or immediately after lambing, the ewes were offered lucerne hay (500 gm), green lucerne (500 gm approx.) and Phosvita 15 gm. Water was freely available except before weighing. Samples of jugular blood (5 ml) were withdrawn from each sheep at 8 a.m. before feeding.

Analytical

Blood ketones.—Acetone, acetoacetic and beta-hydroxybutyric acids were determined by the method of Procos (1961).

Blood glucose.—Glucose was estimated by Lehmann & Silk's (1952) modification of the Folin & Wu (1920) method.

Statistical

(*a*) *Blood glucose.*—The difference between the subgroups fed the green lucerne diet and those fed the lucerne hay diet in respect of blood glucose values during both the pre-fasting and the fasting periods was tested by the method of Danford, Hughes & McNee (1960), since inspection of the values showed that the assumptions of these workers, including homogenicity of the observations on the different days, applied.

(*b*) *Blood ketones.*—The difference between the rates of increase of the ketone body values during fasting was examined by the method of Steel & Torrie (1960), in which the ketone body values were plotted against the square root transformations of the corresponding days of starvation.

RESULTS

Gestation period and lamb production

The length of the gestation period of the Karoo ewes (av. 145, range 141 to 146 days) was slightly shorter than that of the Highveld ewes (av. 146, range 143 to 149 days), and also shorter than that of ewe 34 fasted at 100 days of pregnancy. The Karoo ewes all gave birth with difficulty and tended to retain their placentas, in some cases for two days, while the Highveld ewes lambed normally.

Only ewe 4 from the Karoo bore twins. At birth the lambs of all but one of the twelve Karoo ewes were weak and lacked the desire to suck; the single exception was a strong lively lamb born to ewe 1. By contrast the lambs of six of the Highveld ewes (22, 28, 33, 34, 37, 42) were strong and lively, while those of four (20,25, 51, 53) were weak, and those of two (14, 46) were born dead.

Development of clinical symptoms

Clinical symptoms of pregnancy disease were observed in six of the fasted Karoo ewes but in none of the Highveld ewes. One of the latter collapsed after fasting due to complications at parturition, but this animal showed neither the clinical symptoms, nor the typical fatty changes of the liver associated with the disorder on *post mortem* examination. Of the former, two (1, 6) showed mild and transitory nervous symptoms including any of the following: intermittent tremor, excitability, grinding of the teeth, ataxia and anorexia; three (3, 7, 10) exhibited severe and prolonged nervous symptoms including intermittent twitching, opisthotonus and semi-comatose prostration; and one (5) displayed only lethargy and anorexia before passing into a state of semi-comatose prostration. The prostrate sheep did not recover, and all of them showed fatty changes in the liver on *post mortem* examination (Table 1). It is noteworthy that the mild cases had been fed the green lucerne diet, while the severe and fatal cases had been given the lucerne hay diet in the prefasting period.

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TABLE 1.—*Clinical symptoms and post mortem results of fasting preparturient ewes from the Karoo*

Days of starvation	Lucerne hay prefasting diet				Green lucerne prefasting diet	
	Ewes: 3	5	7	10	1	6
4.....	Normal.....	Normal.....	Normal.....	Normal.....	Normal.....	Intermittent tremor
4.....	Lambed; retained placenta; ataxia	Normal.....	Normal.....	Normal.....	Intermittent tremor	Lambed; ataxia; eating well
6.....	Anorexia; opisthotonus; semi-comatose prostration	Ataxia.....	Excitability; grinding of the teeth	Normal.....	Lambed; anorexia	Normal
7.....	Intermittent twitching; *in extremis*	Ataxia.....	Intermittent twitching	Intermittent twitching; opisthotonus	Eating well.....	Normal
8.....	Dead; *P.M.:* mild fatty changes of the liver	Lethargy; anorexia	Semi - comatose prostration	Semi - comatose prostration	Normal.....	Normal
Post-fasting.....		Opisthotonus; semi - comatose prostration; *in extremis; P.M.:* severe fatty changes of the liver	Dead; *P.M.:* severe fatty changes of the liver	*In extremis; P.M.:* moderate fatty changes of the liver	Normal.....	Normal

The effect of fasting and diet on the live weights of the ewes

At the start of the experiment the animals from the Karoo weighed more (av. 39·0, range 34·7 to 46 kg) than those from the Highveld (av. 33·6, range 28·6 to 39·0 kg). However, in the four weeks during which these animals received the pre-fasting diets, the latter gained more weight than the former. Thus the average weight (39·5 kg) of the Highveld sheep was of the same order as that (39·2 kg) of the Karoo sheep just before fasting. On starvation for eight days the Karoo animals lost less weight (av. 4·6, range 3·4 to 5·4 kg) than the Highveld animals (av. 6·8, range 5·4 to 10·0 kg) irrespective of the prefasting diets.

TABLE 2.—*The effect of fasting and diet on the blood glucose levels*

Group	Prefasting levels (mg%)			
	Lucerne hay diet		Green lucerne diet	
	av.	range	av.	range
Karoo	34·0	27·5–50·5	28·6	17·5–34·0
Highveld*	34·0	26·7–40·0	31·2	21·0–37·6
	Fasting levels			
Karoo	22·5	15·0–33·5	23·1	16·0–35·5
Highveld*	19·5	13·3–27·5	21·0	14·1–28·8

* Ewe 34 excluded

The effect of fasting and diet on the blood glucose levels

Table 2 shows that the average fasting blood glucose levels of the animals from the Karoo were higher than those from the Highveld. With two exceptions (8, 13), the minimal fasting levels (15·0 to 23·0 mg per cent) of the Karoo ewes occurred within the first three days of starvation, while those (13·3 to 19·5 mg per cent) of the Highveld ewes took place from the fourth day onward, with two exceptions (14, 51). On resumption of feeding, the levels of all the animals, including those with poor appetites, increased dramatically. Similar increases occurred just before or immediately after parturition.

Animals fed the green lucerne diet tended to display lower prefasting but higher fasting blood glucose levels than those given the lucerne hay diet.

TABLE 3.—*The effect of diet and fasting on the total ketone body levels*

Group	Prefasting levels (mg%)			
	Lucerne hay diet		Green lucerne diet	
	av.	range	av.	range
Karoo	1·65	1·14–2·04	2·44	1·28–4·65
Highveld*	1·65	1·27–2·00	2·71	1·50–3·42
	Fasting levels			
Karoo	12·48	4·65–24·72	13·02	4·65–24·39
Highveld*	12·25	2·64–23·92	14·27	4·29–28·53

* Ewe 34 excluded

The effect of fasting and diet on the total ketone bodies

From Table 3 it can be seen that the average blood ketone levels of the Karoo ewes were very similar to those of Highveld ewes which had received similar treatments. On the first day of fasting the increase in the ketone levels of the animals from the Karoo (av. 7·93, range 4·65 to 13·67 mg per cent) was greater than that of those from the Highveld (av. 5·56, range 2·64 to 9·61 mg per cent), but from there on the rate of increase in the ketone levels of the latter was greater than that of the former. Thus the maximum levels occurring from the fourth day onward (1, 6 excepted), were higher in the case of the Highveld ewes (14·86 to 28·53 mg per cent) than of the Karoo ewes (11·19 to 24·72 mg per cent). On resumption of feeding the levels returned to normal within two to three days.

Feeding the green lucerne diet in the prefasting period appeared to slow down the rate at which the ketone bodies increased during the fasting period. Thus the rates in mg per cent per day at which the ketone levels increased during the fasting period were 0·83 and 1·81 for Karoo ewes, and 1·54 and 2·39 for Highveld ewes fed prefasting diets containing green lucerne and lucerne hay respectively.

TABLE 4.—*The effect of fasting on the individual ketone bodies*

Group	Prefasting percentages					
	Acetone		Beta-hydroxybutyric acid		Acetoacetic acid	
	av.	range	av.	range	av.	range
Karoo	<1	—	83	75–95	16	4–42
Highveld*	5	0–16	76	62–84	19	7–31
	Fasting percentages					
Karoo	59	28–74	28	16–55	13	9–25
Highveld*	56	32–97	26	0–47	18	3–39

* Ewe 34 excluded

The effect of fasting on the individual ketone bodies

Table 4 summarizes the percentage of acetone, betahydroxybutyric and acetoacetic acids in the blood of ewes from the Karoo and the Highveld. Neither diet nor region of origin of the animals appeared to have any effect on the percentage composition of the individual ketone bodies during either the prefasting or the starvation periods. During the prefasting stage the percentage of the different ketone bodies occurred in this order: beta-hydroxybutyric acid > acetoacetic acid > acetone. On fasting the order changed to acetone > beta-hydroxybutyric acid > acetoacetic acid, the acetone accounting for more than 50 per cent of the total ketone bodies.

Discussion

The Merino ewes from the Karoo region had a greater tendency to succumb to pregnancy ketosis than those from the Highveld. These findings clearly indicate that the susceptibility to this metabolic disorder of animals subjected to exactly the same experimental treatment, after having been kept under identical conditions for six months immediately prior to experimentation, depended on their condition of management prior to this time. This could have been due to their nutritional status in their previous environment or other factors.

Prolonged starvation induced in the preparturient ewes from both regions a hyperketonaemia and a hypoglycaemia, but nervous symptoms including grinding of the teeth, anorexia, ataxia, and semi-comatose prostration described by Van Rensburg (1931), appeared only among animals from the Karoo. This was in spite of the fact that the maximum ketone body levels (14·86 to 28·53 mg per cent) of the Highveld ewes were higher than those (11·19 to 24·72 mg per cent) of the Karoo ewes, while the minimum blood glucose levels (13·3 to 19·5 mg per cent) of the former were lower than those (15·0 to 23·0 mg per cent) of the latter. The clinical symptoms which occurred in six out of twelve animals of the Karoo group were those associated with the subacute form of the disorder (Reid, 1960 a), and the accompanying hypoglycaemic ketosis could just qualify as severe (Reid & Hogan, 1959), since the maximum ketone bodies of the affected animals were over 20 mg per cent, while the minimum blood glucose levels fell within the accepted range (10 to 30 mg per cent).

The fact that the neurological signs occurred only among the Karoo animals whose minimum blood glucose values were higher than those of the Highveld ewes, indicates that the cerebral depression of the affected cases was due to impaired metabolism of glucose which constitutes an obligatory energy source for cerebral tissues (Fazekas, 1958), rather than to the hypoglycaemia *per se*. Most sheep from the Karoo exhibit certain, persistent, sub-clinical, fundamental, biochemical lesions (Wagner, 1964) associated with enzootic icterus and geeldikkop which are endemic in that region (Brown, 1963). Thus the fact that Brown (1964) demonstrated, at least for geeldikkop, not only a substantially retarded rate of clearance of intravenously administered glucose from the circulation, but also a greatly reduced activity of the glyceralaldehyde phosphate dehydrogenase of the Embden-Meyerhof pathway of glucose degradation, favours the above hypothesis. This hypothesis is also in line with the views held by Reid (1960 b): that the metabolic abnormalities of cases of ovine pregnancy toxaemia are consistent with those characteristic of a diabetic-like syndrome in which factors inhibiting glucose utilization may be enhanced under conditions of insulin insufficiency.

In view of the likelihood that production of cellular energy in the Karoo ewes was sub-normal due to biochemical lesions arising from their exposure to the factors causing geeldikkop (Brown, 1964), it is tempting to postulate that these animals lost only two-thirds as much weight as the Highveld ewes during fasting owing to inhibition of energy-producing mechanisms. The mechanisms involving the production of acetyl-Co-A did not seem to be affected, since the composition of the individual ketone bodies either before or during fasting were similar for both groups of animals and for wethers previously examined (Procos, 1962 b). The tendency for animals fed the green lucerne diet to display only mild and transitory nervous symptoms cannot as yet be explained, although the value of fresh green lucerne as a pre-lambing diet is well known among farmers of the Western Cape regions bordering on the Karoo. It is thus clear that a great deal more work is required to locate for certain the rate-limiting reactions responsible for the abnormal metabolism in pregnancy ketosis of sheep, and the effect of endocrine secretions on them.

Summary

The blood levels of glucose and ketones during fasting were determined in two groups of young preparturient ewes from the Karoo region of South Africa where pregnancy disease is endemic, and from the Highveld region where this metabolic disorder rarely occurs among sheep. During the fast, clinical symptoms of pregnancy disease were observed in six out of 12 Karoo ewes but in none of the Highveld ewes; the Karoo ewes lost only two-thirds of the weight lost by the Highveld ewes despite the similarity of the starting weights of both groups; the Karoo ewes had higher blood glucose and lower blood ketone levels than the Highveld ewes; in both groups of animals fasting changed the order of magnitude of the individual ketone bodies from beta-hydroxybutyric acid > acetoacetic acid > acetone to acetone > beta-hydroxybutyric acid > acetoacetic acid. The effect of feeding green lucerne in place of lucerne hay in the prefasting diet was to raise the fasting blood glucose levels, to lower the rate of increase in the fasting blood ketone levels, and to reduce greatly the incidence and intensity of the clinical symptoms. The findings clearly indicated that the susceptibility to pregnancy disease of animals subjected to exactly the same experimental treatment, after having been kept under identical conditions for six months immediately prior to experimentation, depended on their condition prior to this time. The possibility that the symptoms of cerebral dysfunction were due to impaired glucose metabolism often associated with Karoo sheep rather than to the hypoglycaemia *per se* is discussed.

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