ANIMAL FERTILITY: A POSSIBILITY FOR BIOLOGICAL QUALITY-ASSAY OF FODDER AND FEEDS?[1]

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With the increasing alterations being brought about today within plant and animal environments as a result of agricultural intensification, the development of quality testing methods for fodder and feeds is of rising significance. The important inorganic constitutents of a product are represented currently through chemical and biochemical analyses, but hardly the organic; as to the interacting of the contents and their effect on the animal organism, relatively little is known. Consequently, the search for measurable and meaningful characteristics of quality is of special urgency.

Observations on Breeding Bulls

It has long been known that sperm glands are especially sensitive to environmental stress. As early as 1962, considerable individually-varying damage to the sperm quality of breeding bulls, due to deficient fodder, was reported. (Aehnelt and Hahn, 1962) The significance of environmental stress with regard to fertility is especially recognizable in comparative studies of the progeny of seeding bulls. For example, Hahn (1962) detailed cases of 30 bull-daughter groups of the German Black-spotted cow (a total of 1,962 heifers) showing, under favorable environmental conditions, an average first fertilization take of 84.4% non-return, whereas in contrast the groups under unfavorable environments gave a corresponding average of only 56.5% (29.4 to 72.7% in the single progeny groups). Similar differences were also described by Lotthamer (1967) with the Spotted and Yellow cow.

In 1963, one breeding station published a report on high-level *functional breakdown of the testicles* within breeding bulls gathered over a period of several years. These particular bulls had all been receiving fodder from a farm implementing very high and

[1]Summarization of previously reported findings. First published in *Tierärztliche Umschau*, No. 4, 1973, Constance, Switzerland. Re-published in *Lebendige Erde*, No. 3, 1974, Darmstadt, Germany. Permission for the English printing was kindly given by Dr. H. Heinze. Trans. by W. F. Brinton.

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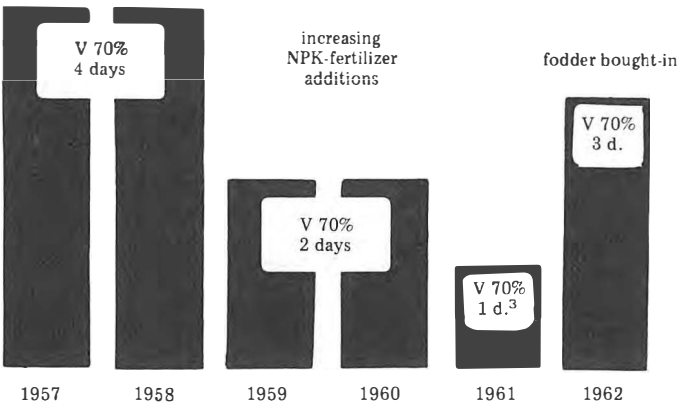
increasing levels of chemical fertilization (up to 200 lb N, 100 lb P_2O_5 and 200 lb K_2O per surface fodder-acre). Concurrent with this farm's intensification, the average "fertilization capacity" of the diluted ejaculate (= the maintenance of 70% forward movement of the sperm cells) fell from over 4 days as it had been in the years 1957 and 1958 to just over 2 days in 1959 and 1960 and finally to around 1 day by 1961 (Diagram 1).

Diagram 1



Average sperm quality (maintenance of 70% forward movement in the years 1957 to 1962) of 7 bulls on a breeding station receiving fodder from a farm with marked intensification.

Furthermore, in the course of 1961, two bulls had to be eradicated due to nectosis of the testicles with aspermie. After this station converted in 1962 to using more "extensively" produced fodder, the average "fertilization capacity" of similar ejaculates rose again to around 3 days. In order that other causes (such as virus infections) could be demonstrably ruled out on the basis of a comprehensive study, the question of a connection between reproductive impairment and agricultural intensification was taken on (Aehnelt and Dittmar, 1963).

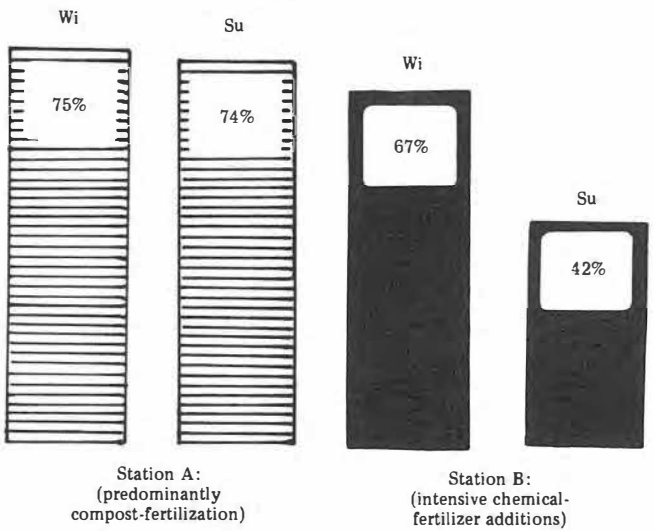
We also investigated the *different fertility levels of bulls from two comparable breeding stations.* The pasture land of both stations had been very differently treated for five years, one with chemical fertilizer additions of 120 lb N, 60 lb P_2O_5 and 100 lb K_2O per acre, including trace minerals, while the other made use of manure compost predominately. The fertilization scheme on the

[3]Two bulls lost to testicle nectosis and aspermie.

"intensive farm" was managed exclusively on the basis of complete soil analyses performed by an experienced institute.

The consistency of the testicle tissue and the sperm quality of the bulls kept on the station using compost applications was clearly superior. During summer pasturing, this station showed 74% of all diluted ejaculates having forward movement of sperm cells on the 4th day, with a minimum of 70%. In contrast, a corresponding average of only 42% was revealed on the station employing mineral fertilization but otherwise using an identical animal treatment and feeding scheme.

Diagram 2



Sperm quality of bulls from 2 breeding stations implementing different management. Portion of the diluted ejaculates (altogether 625) showing 70% forward movement on the 4th day of preparation under refrigeration.
Wi = winter; Su = summer

Moreover, the yearly decline of bulls due to insufficient fertility was essentially greater on the "intensive farm" than on the "compost farm". During the six-month summer, the feed of the bulls came mainly from each station's own pasture. In the winter, insofar as the animals on the chemically fertilized intensive farm received, in addition to the station's own hay, good, herb-rich mountain hay as well as a more diverse feed ration concentrate (esp. grain meal and clover), the differences in sperm quality between the two stations were essentially lesser. In total, 625 ejaculates were evaluated (Aehnelt and Hahn, 1965).

Experimental Investigations with Rabbits

(1) RESEARCH ANIMALS AND RESEARCH LAY-OUT

On the basis of these prior observations, as well as on numerous other accounts, it was our opinion that a feeding experiment using variously grown fodder was necessary. Due to the fact that no means were available to perform such exact studies with large animals such as cattle, an experiment making use of rabbits was worked out in the last years. Here, genetically defined, parasite-free animals, in groups of 5-10, received over a period of 6 weeks previously examined fodder — 150 to 200g dry fodder and approximately 500g moist fodder per animal per day. The fresh fodder was complemented with 50g pelleted feed. The particular rabbits used were predominately F_1-animals of the Black Alaskan x White Wiener breed, aged roughly 5 months and of an average weight of 3kg (Breeders: Institute for Experimental Animal Breeds TNO, Zeist/Holland).

At the conclusion of the feeding period, the rabbits were treated with 2.5mg of a LH-preparation (Pituitary Luteinizing Hormone[4]), fertilized, and on the next day or 26-30 hours later, sacrificed. After gathering the egg cells by means of a washing technique, the ovaries, uterus and kidney were separated and examined macroscopically as well as histologically.

As applicable measurement for fertility, the following criteria have been chosen for this purpose: number of ovulation periods, total egg count and percentage fertilized eggs, capacity for propagation of egg cells *in vitro*, and number of uterine glands in the uterus wall. As a means of checking for kidney stress, the ascorbic acid test has been applied recently (Hahn and Aehnelt, 1972).

(2) EXPERIMENTAL RESULTS

(a) *Unfertilized and Intensively Fertilized Hay*

A first feeding trial, repeated several times with unfertilized and intensively fertilized hay from the same locations, brought interesting results. The "intensive" pasture land had received mineral fertilizer, slurry as well as manure, giving in total 120 lb N, 110 lb P_2O_5, and 400 lb K_2O per acre per year. Within six weeks considerable differences between the animals in the two feeding groups were already noticed. The fertility of the "intensive group" was highly disturbed; a marked atrophy of the uterus was

[4]Produced by Armour-Baldwin Laboratories, Omaha, Nebraska.

recognizable (thickness only 2mm as opposed to the "extensive" hay's 5mm). Moreover, the number of fertilized egg cells in the "intensive" group was reduced to 20% of that of the "extensive" group, and after 24 hours in incubation they were totally degenerated. In a related mouse experiment, sexually active compounds with an anti-estrogenic effect were discovered in "intensive" hay (Hahn et al, 1971).

(b) *Hay from Problem Farms*

In a further study, hay from an extensive bio-dynamic farm — as opposed to hay from two intensive farms — showed itself to be considerably superior with regard to almost all fertility characteristics of the rabbits raised on it (Surveys 1 and 2).

SURVEY 1

Feeding Trial of Rabbits with Hay (Pellets) from Farms with Intensive and Extensive Fertilization (per group of four animals; Duration of 6 weeks)

Fertility Criteria (average values)	*Hay Pellets without Supplementation Experiment Groups*		
	1 *intensive**	2 *intensive**	3 *extensive*
Ovary weight in mg	144	128	233
Ovulation periods, no.	3.8	3.3	8.8
Egg Cells found, no.	1.8	0	8.6
Fertilized Egg Cells in %	0	0	93.1
Uterine Glands**, no.	20.2	21.4	32

*from farms with herd sterility problems in cows
**per equal surface area

In Survey 1, the results of feeding with hay from both intensive farms (Experiment Groups 1 and 2) are compared with the corresponding results with hay from an extensive farm (Group 3). Here the hay was fed without further supplementation. The particular distress reported in the cows on the farms supplying the "intensive" hay is likewise reflected in the rabbit results, for we see marked fertility disturbances in each criterion investigated.

In Survey 2, the same hay samples were used except that now a supplementation was added consisting of 14% Soja bean meal and 6% molasses in the form of pellets. Hay from a bio-dynamic farm served as the control, receiving the same supplementation. Here an improvement in fertility characteristics is observed only in

the case of Group 2, i.e. an intensive group; nevertheless, the fertility level as represented in the control (bio-dynamic) group is not achieved. Moreover, with the help of the ascorbic acid test, a relatively greater kidney stress is revealed in connection with the hay from both intensive farms as opposed to that from bio-dynamic sources (ascorbic acid in the kidneys: 606 and 487 γ in intensive groups and 251 γ in bio-dynamic group).

SURVEY 2

Feeding Trial of Rabbits Receiving Hay from Farms of Different Management (per group of four animals; Duration 6 weeks)

Fertility Criteria (average values)	*Hay Pellets plus 14% Soja Bean and 6% Molasses Experiment Groups*		
	1 *intensive**	2 *intensive**	3 *OG-bio-dynamic*
Ovary weight in mg	156	227	271
Ovulation periods, no.	3.5	6.0	9.3
Egg cells found, no.	3	6	9
Fertilized egg cells in %	0	100	96
Uterine glands**, no.	28.5	25.7	100
Ascorbic acid Kidney test, γ	606	487	251

*from farms experiencing herd sterility in cows
**per equal surface area

Those fields producing the hay on the two intensive farms were fertilized with 280 lb N, 240 lb P_2O_5, and 240 lb K_2O (Group 1) and 280 lb N, 90 lb P_2O_5 and 340 lb K_2O (Group 2) per acre per year, a mixture of slurry, liquid manure, farmyard manure and chemicals. Noteworthy is the especially high P-fertilization and relatively greater share of chemicals in the total amount of minerals put down in the intensive group which compared least favorably. In this Group (1), of the total amount of minerals applied, 125 lb N, and 216 lb P_2O_5 were in the form of chemical fertilizer as opposed to 35 lb N and 29 lb P_2O_5 in Group 2. The "extensive" hay was fertilized exclusively with approximately 1,100 gallons liquid manure which supplied, according to Hahn and Lengauer, about 27 lb N, 12 lb P_2O_5 and 25 lb K_2O per acre. The bio-dynamic hay received only compost; however an accurate reckoning of the amount of minerals applied thereby is not possible here.

(c) *Potassium Feeding Experiment*

Within the context of the rabbit experiments, a feeding trial with potassium increments was also carried out (K-content of fodder used is 1.10%; 2.60%; 3.90% — see Survey 3). The addition was in the form of potassium chloride. Unfortunately for the experiment, no uniform batch of animals was available so that consequently the fluctuation between the animals was relatively high. Since only four animals were used per group, the results of this experiment are to be interpreted with care (see Maercklin, 1971). Nevertheless, surprisingly similar tendencies are revealed in all three groups, indicating a decrease in fertility concurrent with increasing levels of potassium in the fodder. In the two extreme groups of this experiment (1.10% and 3.90% K), the following comparative values are found, respectively: fertilized egg cells, 100% vs. 56%; development of egg cells *in vitro* until the morula stage, 100% vs. 20%; number of uterine glands (per equal surface area), 45 vs. 21. The number of ovulation periods revealed no differences. Furthermore, atrophied conditions in the kidneys were evident in connection with the higher K-content (Hahn et al, 1972).

SURVEY 3

Fertility and Kidney Results (Average Values)
in Rabbits Receiving Fodder (Prepared Ration)
with Varying Potassium Additions
(per group of 4 animals; Duration 6 weeks)

	Experiment-Groups		
	1	2	3
K in fodder in %	1.10	2.60	3.90
Ovulation periods (no.)	4.7	5.7	3.9
Fertilized egg cells in %	100	73	56
Morula *in vitro* in %	100	82	20
Uterine Glands (no.)*	45	34	21
Kidney weight in mg	267	236	182
Kidney surface/animal**	205	145	146

*per equal surface area
**determined with planimeter instrument

(d) *Carrot Experiment*

Going further, a feeding experiment with carrots grown under various types of fertilization was undertaken. Considered

here are two carrot crops grown with chemical-mineral fertilization per acre of 1) 300 lb N, 100 lb P_2O_5, and 180 lb K_2O; and 2) 100 lb N, 100 lb P_2O_5, 180 lb K_2O; and finally 3) a crop grown according to bio-dynamic methods (see Survey 4).

SURVEY 4

Feeding Trial of Rabbits with Differently Grown Carrots
(per group of 4 rabbits; Trial duration 5 weeks)

	Experiment Group		
	1	2	3
Fertilization	*mineral-NPK with 300 lb N/a*	*mineral-NPK with 100 lb N/a*	*Organic *bio-dynamic*
Uterus musculature in cm*	3.1	6.0	7.3
Uterine glands** (average no/animal)	19	31	42
Ovulation periods (average no. animal)	6.2	10.2	11.7
Egg Cells found (average no/animal)	4.8	5.7	11
Fertilized egg cells in %	37	52	52

*on projected surface, average values
**per equal surface area

The fertility characteristics of the 300 lb N/a Group were about 50% lower than those of the bio-dynamic group, which gave the best results. Between these two groups the following average values were observed, respectively: uterus musculature, 3.1 cm vs. 7.3 cm; number of uterine glands per unity surface area, 19 vs. 42; number of egg cells found, 4.8 vs. 11; fertilized egg cells, 37% vs. 52%. The bio-dynamic group was also superior to that of the 100 lb N group on 2 essential fertility counts; number of egg cells and uterine glands, the latter being significant for the nutrition of the embryo.

Noteworthy are the observable differences despite the relatively short experiment duration of only 5 weeks. Each rabbit received daily approximately 500g (1 lb) carrots plus 50g prepared feed. The feed-concentrate rate was added on grounds of completing the ration and has probably had the effect of balancing out somewhat the unfavorable influences of the "intensive carrots" and consequently diminishing the differences between all 3 groups.

(e) *Kohlrabi Experiment*

Finally, results rich with information were had from a kohlrabi feeding trial (Survey 5).

SURVEY 5

Feeding Experiment of Rabbits with Differently Grown Kohlrabi
(per group of 5 rabbits; Experiment duration 5 weeks)

Fertilization	*Experiment Group* 1 *highly chemical**	2 *Organic - bio-dynamic*
Ovulation periods (average no/animal)	7.6 (uncertain)	8.2
Egg Cells found (average no/animal)	4.6	5.6
Fertilized egg cells in %	18.0	83.0
Blastocytes in % after 4-day cultivation	0 (degenerated after 24 hours)	77.0
Vitamin C in ovaries (in γ)	44.4	13.5

*average 225 lb N, 160 lb P_2O_5, 240 lb K_2O/acre

With high mineral fertilization (Group 1) the number of fertilized egg cells was much lower than that of the bio-dynamic group. Moreover, 77% of the egg cells in the bio-dynamic group developed *in vitro* until the blastocyte stage, whereas in contrast every single egg cell in Group 1 degenerated after 1 day. The higher Vitamin C content in the ovaries of the "intensive group" in comparison to the "bio-dynamic group" points to great and prolonged stress with subsequent appearances of exhaustion in the organs.

Discussion of Results

The results of the experiments carried out indicate that with increasing fertilization of fodder and vegetables alterations can take place within the plants which, upon subsequent feeding, lead to disturbances in animal reproduction. The field observations mentioned in the beginning of this report concerning breeding bulls, and other numerous examples of herd damage resulting from intensification, are borne out here (Aehnelt and Konermann, 1961; Schiller et al, 1962, 1967, 1968; Romanowski, 1966; Seekles, 1969; Hermisson, 1970 and Zacharias, 1970). Although

these rabbit experiment results cannot be simply extrapolated to cattle, they are, nonetheless, surprising with regard to the nature of widespread, similarly expressed upsets in connection with agricultural intensification.

Aside from chemical fertilizers, non-biologically prepared organic fertilizers (especially slurry and liquid manure) in massive quantities can also exert unfavorable influences. Whether and to what extent plant protective sprays are significant here cannot be gathered from the foregoing results.

These findings are especially remarkable insofar as the rabbits had received the "trial-fodder" for a period of only 5-6 weeks. Moreover, with only one exception (Survey 1) all the experimental feedings had an additional fodder supplementation (soja bean-meal and molasses plus a diverse concentrate). Due to this a certain diminishing of the "damaging influences" of the trial fodders and consequently a leveling out of the results were to be expected.

The results described must nevertheless be interpreted carefully, since relatively few trials and experimental animals were involved. A *comprehensive inquiry and completion of the foregoing results* is without question required. The question to what extent agricultural intensification is possible without damaging effects should be investigated. For further research in this direction the following measures are planned:

1. A greater number of experimental animals should be used and more parallel experiments undertaken.
2. The feeding trials should be applied to other animals as well, and to smaller laboratory animals as well as to cattle.
3. Aside from examining reproductive organs and kidneys, other organs such as the pituitary gland and liver should be taken. Biochemical analyses are also contemplated.
4. The biological assay should be, if at all possible, extended to a variety of feeds and fodder.
5. Intended is that the raising of experimental feeds (especially green fodder, hay, various vegetables and grains) be arranged on an increment basis with increasing application levels of NPK in mineral form.
6. In connection with the last point it is also foreseen that various organic fertilizers (especially slurry, liquid manure and compost) and conventional plant-protective sprays be likewise investigated on an increment basis.

The goal here is the mediation of an optimal fertilization in order that optimal quality and optimal yields be made possible.

The preceding results indicate that the rabbits' reproductive system may be applicable as a biological indicator in checking for nutritional upsets and thereby for *investigating nutritive quality* (Hahn and Aehnelt, 1972).

In concluding, let it be mentioned that the experiments discussed have been a joint Austrian-German project. The cultivation, harvesting and preparation of the previously-analyzed fodders, as well as the soil and feed analyses, were directed and carried out by either the Federal Agricultural Experiment Station in Linz, Austria (Director: Dr. Schiller) or the Training and Experimental Station for Greenland Management, Fodder Production and Landscape Design in Bredstedt/Schlesw.-Holst. (Leader: Dr. Bracker). Here we would like to express our heartfelt thanks to both these institutes for their trustworthy and harmonious work together.

Due to lack of space, the data from the detailed analyses referred to will not be disclosed. Noteworthy nevertheless is the fact that despite the different fertilization of the various fodders, their nutritional and mineral contents varied only slightly. Because of this, it is conjectured that, concurrent to rising agricultural intensification, previously unknown, organic plant compounds with unfavorable sexual influences may be produced or rendered active.

Summary

1. Feeding experiments with rabbits receiving intensively fertilized hay, carrots and kohlrabi, plus concentrates with high levels of potassium, were carried out for a period of 5-6 weeks. Consequently, it was demonstrated that obvious disturbances of reproductive traits and partially increased kidney stress with the appearance of exhaustion occurred.
2. As controls, comparable fodder grown either "extensively" or bio-dynamically was used. These gave as good results in general as a ready-made diverse rabbit ration.
3. According to the current state of our research, the following fertility criteria are of significance: number of ovulation periods plus egg cells found and the proportion of them fertilized; reproductive capacity of the egg cells *in vitro;* number of uterine glands, and the ascorbic acid test for kidney stress.
4. The foregoing findings with rabbits must be investigated in the context of a more extensive project. Aside from this, experiments with smaller as well as larger animals are contemplated.

5. The trials should be extended to embrace other fodder and feeds including vegetables and grains.
6. These results have shown that rabbit reproduction may be used as a biological assay technique for fodder and feeds. Such investigations may be also be significant insofar as they delineate an existing border line with regard to agricultural intensification.

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