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Effects of dietary organic selenium and vitamin E supplementation on post mortem oxidative deterioration in muscles of pigs

Dedicated to Professor Dr. Erhard Kallweit on the occasion of his 65th birthday

Summary

The effect of feeding high levels of α-tocopherol and organic selenium (Se) to pigs on colour stability and on the susceptibility to oxidative deterioration was investigated. Treatments consisted of supplementation of vitamin E (200 mg/kg diet), organic Se (0.3 mg/kg diet) and both vitamin E and organic Se for the last 60 days to finishing pigs before slaughtering. *Longissimus dorsi* (LD) and *psoas major* (PM) muscles were examined after 2 and 7 days of storage on colour stability and on lipid peroxidation (measured as malondialdehyde equivalents). Rate of oxidation by stimulation with Fe^{2+}/ascorbate was also estimated in LD samples obtained post mortem. In PM 7 days post mortem we found differences between control and groups of pigs supplemented with vitamin E and Se on reflectance, but significant differences ($P<0.05$) were found only in the vitamin E + Se group.
Positive effects ($P<0.05$) of vitamin E and Se on colour in *psoas major* muscle refrigerated for 7 days are supported with significant ($P<0.05$) lower levels of TBARS values in pigs supplemented with vitamin E and organic Se as well. Supplementation with organic Se does not affect the oxidative stability of muscle tissue (*longissimus dorsi*) when the rate of iron-induced lipid oxidation was examined.
Dietary Se had limited potential for enhancing the quality of pork carcasses (*psoas major*) and accentuating the effect of vitamin E on the oxidative stability of *longissimus dorsi* muscle was not found.

Key Words: pig, vitamin E, selen, oxidative stability, colour, musculus *longissimus dorsi, psoas major*

Zusammenfassung

Titel der Arbeit: **Einfluss einer Futtersupplementierung mit organischem Selen und Vitamin E auf postmortale oxidative Veränderungen im Schweinemuskel**
Die Auswirkung eines hohen Gehaltes an α-Tocopherol und organischem Selen im Futter auf die Farbstabilität und auf oxidative Veränderungen des Fleisches beim Schwein wurden untersucht. 60 Tage vor der Schlachtung wurde das Futter mit Vitamin E (200 mg/kg) oder organischem Selen (0,3 mg/kg) oder mit beidem angereichert. Die Farbstabilität und die Lipidperoxidation der Muskeln *M. longissimus dorsi* (LD) und *M. psoas major* (PM) wurden nach zwei und nach 7 Tagen Lagerung ermittelt. Ebenfalls bestimmt wurde die Rate der Peroxidation nach Einwirkung von Fe^{2+}/Ascorbat. Nach 7 Tagen Lagerung unterschieden sich die Reflexionswerte im PM zwischen der Kontrollgruppe und der Vitamin E und Selen Gruppe. Dieser positive Einfluss der Futtersupplementierung führte auch zu einer geringeren Konzentration an thiobarbitursäurereaktiven Substanzen. Die Futteranreicherung mit organischem Selen allein hatte keinen Einfluss auf die oxidative Stabilität.
Während Vitamin E eine günstige Wirkung auf die Qualität zeigte, bewirkte Selen keine Verbesserung der Fleischqualität (PM) und beeinflusste auch nicht den Effekt von Vitamin E auf die oxidative Stabilität (LD).

Schlüsselwörter: Schwein, Vitamin E, Selen, oxidative Stabilität, Farbe, *M. longissimus dorsi, M. psoas major*

I. Introduction

Oxidative rancidity of muscle systems begins shortly after death and involves the production of a complex mixture of aldehydes, ketones and alcohols. Non-ham iron, trace elements, and sodium chloride added to processed meat products initiate pre-

ferable the oxidation of highly unsaturated phospholipids. α-tocopherol, when incorporated into animal diets, is a highly effective lipid-soluble chain-breaking antioxidant which is acceptable to the consumer (FAUSTMAN et al., 1989). Vitamin E and selenium (Se) are essential nutritients that are integral components of the antioxidant defence system of cells and tissues.

The beneficial effect of vitamin E supplementation on meat quality characteristics is related to lower lipid oxidation (MONAHAN et al., 1992), enhanced colour stability (FAUSTMAN et al., 1989), benefical effects on muscle energetic metabolism, electrical conductivity, drip loss (LAHUCKY et al., 2000) and enhanced the possible storage time of the products (BUCKLEY et al., 1995).

The formation of metmyoglobin (MetMb) from oxymyoglobin (OxyMb) is positively correlated to lipid oxidation and appears to be dependent on antioxidant status (YIN et al., 1993). Free radicals produced during lipid oxidation can alter the heme chemistry and initiate pigment oxidation causing loss of desirable colour (MIKKELSEN et al., 1992).

Selenium was earlier identified as an integral part of the enzyme glutathione peroxidase (GSH-Px), which destroys lipid peroxides and functions by protecting of cell membranes against peroxidative damage (HOEKSTRA, 1975). Later, selenium was identified as a component of 5´-iodothyronine deiodinase which converts tetra- to triiodothyronine and functions in nonshivering thermoregulation (BEHNE et al., 1992). GSH-Px activity is concidered one of the best indices of selenium status and utilisation (SANKARI, 1985). It has the advantage that it is not affected directly by vitamin E and can be measured quantitatively (for review see MILAD and KOVAC, 1998). Serum glutathione peroxidase activity generally reached a plateau at a dietary level of 0.1 ppm Se when either inorganic or organic Se sources were supplemented to growing or finishing pig diets, but the Se enriched yeast source seemed to be less biologically available (MAHAN and PARRET, 1996). The .effects of organic and inorganic Se on pigs have been studied widely (GOEHRING et al., 1984; KURKELA and KÄÄNTEE, 1984; MAHAN and PARRET, 1996). No significant differences were found in daily gain, feed/gain ratio or carcass quality of growing pigs fed with 0.1 mg inorganic Se or the same amount of Se bound in yeast (SUOMI and ALAVIUHKOLA, 1992). Selenium in meat of pigs may also contribute to the solution of a sufficient supply of this element into the human organism (KOUTNIK and INGR, 1998). From the literature available it is not yet enough clear if organic bound Se could improve vitamin E effects on properties of meat.

The objective of this study was to evaluate the effects of dietary administration of vitamin E alone and in combination with organic bound Se on oxidative stability of muscle tissue of the porcine carcass.

2. Material and methods

2.1. Animals and diets

Forty Large White x Pietrain were used in this experiment, including 30 gilts and 10 castrates. The RYR-1 genotype of these animals was determined by a DNA based test (Genetic department, RIAP Nitra) described previously (LAHUCKY et al., 1998). To

create homogenity of groups on frequency occurence of mutation on RYR-1 gene and on sex of pigs four groups were formed with 5 normal and 5 heterozygotes on MH syndrome. The pigs were penned in double boxes at institute facilities to minimise the influence of stress. One group was fed a diet (not supplemented with vitamin E) and the other groups received a supplemental level of vitamin E (200 mg kg^{-1}), organic bound selenium (0.3 mg kg^{-1}), and a mix of vitamin E (200 mg kg^{-1}) and organic selenium (0.3 mg kg^{-1}) for 60 days before slaughtered. The vitamin E was provided by Slovakopharma (Hlohovec, Slovakia) and organic selenium (Sel-Plex 50) by Alltech. Differences on MH genotypes and sexes were not evaluated.

2.2. Sample collection and chemical analysis

Animals were stunned, slaughtered and exsanguinated in the slaughter house of RIAP Nitra (transportation about 200 m) with a mean live weight of 113 kg. Longissimus muscle samples were collected immediately after exsanguination (0 h), frozen in liquid nitrogen and stored at - 70° C until used for estimating of oxidative stability. Following slaughter, the carcasses were chilled at 4° C for 24 h after which *longissimus dorsi* (LD) and *psoas major* (PM) muscles were removed from each carcass. A portion of the sample was used immediately and the remaining sample was wrapped in aluminium film and stored in a refrigerator at 4° C for 7 days. A portion of LD was also frozen in liquid nitrogen and stored until analysed.

Vitamin E. The concentration of vitamin E in muscle were measured by HPLC (BERLIN et al., 1994). A mixture of 1.5 ml muscle homogenate, 2 ml absolute ethanol and 0.5 ml 10 % ascorbic acid was heated to 70°C for 5 minutes. After adding 1 ml 10 N KOH, the mixture was incubated at 70°C for 30 minutes. After cooling, 5 ml n-hexane was added for extraction. The solvent was removed by evaporation under nitrogen, and the vitamin E was immediately resolved in absolute ethanol and assayed by HPLC. HPLC analysis was performed with the mobile phase methanol with a flow rate of 1 ml/min and a Lichrospher RP 18 column with precolumn (Muder & Wocherle Chromatographietechnik Berlin, 12.5 x 0.4 cm, 5 µm). Detection was performed by fluorescence at 292 nm excitation/336 nm emission. Peaks were quantified upon calibration with authentic samples of vitamin E (Sigma, Deisenhofen).

2.3. Meat quality

Colour changes after refrigerated storage were measured on the freshly cut surface of the sample by means of spectrophotometer (Specol, Germany) at 520, 580 and 640 nm as external reflectance. Values at 580 and 640 nm were used for calculation of R (reflectance at 580 - reflectance at 640 nm) which should be related to metmyoglobin production (RENERE et al., 1987). Lipid oxidation was assessed at 24 h and 7 days by the 2-thiobarbituric acid method of SALIH et al. (1987). For evaluating the peroxidative stability of *longissimus* homogenates the determination of thiobarbituric acid reactive substances (TBARS) was used. TBARS were expressed in terms of malondialdehyde, a breakdown product formed during peroxidation. To stimulate lipid peroxidation 3 ml of muscle homogenate were incubated in 0.1 mM ascorbate and 5 µM $FeSO_4$. From this 0.5 ml were immediately removed and pipetted into 0.25 ml of

20 % trichloracetic acid (TCA) in 100 mM KCl. The remaining homogenate was placed in a water bath at 37° C and after 30, 60, and 120 min 0.5 ml each of this incubated homogenate were transferred into the TCA medium (see above). These samples were centrifuged at 10000 g for 10 min and 0.5 ml of the supernatants were mixed with 0.5 ml thiobarbituric acid (0.67 %) and boiled for 15 min in a water bath. The absorbance at 535 nm was determined immediately after cooling. Standard MDA solution was prepared by hydrolysis of 1,1,3,3-tetraethoxypropane and the results were expressed as nM MDA/ mg^{-1} homogenate protein. Protein content of homogenates was estimated by a modified biuret method.

Statistical analyses were calculated as mean values and standard deviation and differences were evaluated by t-test.

Table 1

Groups of supplementation with vitamin E and organic selenium in diet and concentration of vitamin E (α-tocopherol) in *longissimus dorsi* muscle (Fütterungsgruppen und Konzentrationen von Vitamin E im *M. l. d.)*

Group		Vitamin E (mg/kg^{-1} diet)	Se (mg/kg^{-1} diet)	Vitamin E (LD) (mg/kg^{-1})
Control	mean	0	0	0.45^a
(n=10)	s.d.			0.144
Vitamine E	mean	200	0	1.23^b
(n=10)	s.d.			0.317
Se	mean	0	0.3	0.543^a
(n=10)	s.d.			0.202
Vitamin E +Se	mean	200	0.3	1.16^b
(n=10)	s.d.			0.66

Means with different superscripts differ ($P < 0.001$)

Table 2

Colour measured as reflectances and amount of thiobarbituric acid (TBA) related compounds in muscles *longissimus dorsi* (LD) and *psoas major* (PM) of pigs during chill storage (Reflexionswerte und thiobarbitursäurereaktive Substanzen im *M. longissimus dorsi* und *M. psoas major* nach Lagerung)

Values/Group day	Day	Control mean	s.d.	Vitamin E mean	s.d.	Se mean	s.d.	Vitamin E + Se mean	s.d.
Colour (Reflectance, %)									
Longissimus dorsi									
Reflectance (R) 580 nm	2	19.65	4.816	21.91	3.863	21.54	2.797	21.87	7.745
630 nm	2	44.78	5.031	45.94	5.145	44.73	4.484	47.02	9.496
Difference (R580 - R630)	2	25.13	5.003	23.70	2.997	23.98	4.745	25.15	4.349
Reflectance 580 nm	7	21.50	4.594	23.16	3.634	22.09	4.136	22.91	4.009
630 nm	7	47.03	6.315	48.17	6.554	46.06	5.942	44.93	7.674
Difference (R580 - R630)	7	25.53	5.499	25.04	4.327	23.97	4.094	22.02	6.108
Psoas major									
Reflectance 580 nm	2	12.85	2.684	14.69	2.997	13.37	2.546	15.12	3.330
630 nm	2	39.20	4.725	43.12	5.986	46.06	5.942	44.93	7.674
Difference (R580 - R 630)	2	26.35	3.571	28.43	4.910	25.69	4.746	25.84	3.004
Reflectance 580 nm	7	13.97	2.878	14.44	2.978	14.25	2.768	14.41	3.267
630 nm	7	43.57^a	3.833	41.33	5.908	39.58	6.788	40.41^b	3.978
Difference R(580 - R630)	7	29.53	5.499	26.88^b	3.539	25.33^b	4.906	26.00^b	3.762
TBARS values (mg/kg^{-1})									
Longissimus dorsi									
	2	0.26	0.103	0.24	0.067	0.21	0.076	0.24	0.072
	7	0.78^1	0.185	0.75^2	0.213	0.70^1	0.250	0.80^2	0.210
Psoas major									
	2	0.30	0.097	0.26	0.139	0.27	0.100	0.22	0.082
	7	1.02^{3a}	0.114	0.82^{3b}	0.151	0.78^3	0.271	0.74^{3b}	0.214

Means with different superscripts differ ($P < 0.05$) [1] (n=8); [2] (n=7); [3] (n=6)

Results and Discussion

The content of vitamin E (α-tocopherol) of the *longissimus dorsi* muscles was determined and the results are presented in Table 1. The levels of vitamin E in muscles were found to be higher in groups of pigs supplemented with vitamin E and highly correlated with the dietary intake ($P<0.01$). The amount of vitamin E analysed in *longissimus dorsi* in the present study when pigs were fed 200 mg vitamin E/kg feed was more than twice higher if compared to control and with Se supplemented pigs. This is comparable to previously reported results (BUCKLEY et al., 1995). Lower levels of vitamin E in LD in supplemented and control pigs, found in our experiments, if compared to results which were introduced by another authors (MONAHAN et al., 1992; BUCKLEY et al., 1995; HONIKEL et al., 1998; LAURIDSEN et al., 1999) could account for no supplemented vitamin E in basal diet and/or longer time stored and manipulation of samples (6 months stored and once thawed and frozen). The level of vitamin E in *longissimus dorsi* muscle of control and supplemented pigs with our experimental conditions were twice lower as introduced by HONIKEL et al. (1998).

Numerous studies have found dietary vitamin E to improve the colour of chill-stored pork chops (MONAHAN et al., 1992; BUCKLEY et al., 1995). Results vary considerably and findings of non-significant effects of dietary vitamin E on colour stability have also been reported (JENNIES et al., 1997). In the present study by administration of vitamin E and Se colour stability (7 days storage) was unaffected in LD muscle using surface reflectance measurements and difference values (630nm - 580 nm). Using *psoas major* muscle we found differences between control and group of pigs supplemented with vitamin E and Se on reflectance at 630 nm but significant differences ($P<0.05$) were in group vitamin E + Se. It is supposed that higher differences on reflectance values between 630 nm and 580 nm are connected with a higher level of methmyoglobin (RENERE, 1987). We found significant ($P<0.05$) lower differences between reflectance at 630 and 580 nm in *psoas major* if comparing control and with vitamin E and Se administered pigs. Our results are in agreement with findings of no significant changes in colour stability in porcine *longissimus dorsi* (LANARI et al., 1995; JENSEN et al, 1998). When administered vitamin E colour of *psoas major* could be stabilised as was shown mainly in beef (CHAN et al., 1996). Dietary supplementation with vitamin E has been shown to reduce lipid oxidation and accumulation of metmyoglobin in fresh beef (FAUSTMAN et al., 1989). In agreement with our results on *longissimus dorsi* muscle the changes in colour stability as a consequence of feeding high levels of vitamin E in pork is not as evident as in beef. ASGHAR et al. (1991) showed that 'a' values (an indicator of surface redness) of pork chops from pigs fed vitamin E (200 mg/kg feed) were significantly higher than those of chops from pigs fed the basal level (10 mg/kg feed) after 6 days of refrigerated storage. MONAHAN et al. (1992) reported a higher 'a' value in chops from pigs fed the higher level of vitamin E after 2, 4, 6 and 8 days of refrigerated storage. An effect on surface-redness ('a' value) of *biceps femoris* slices from cured hams from pigs supplemented with vitamin E (200 mg/kg^{-1} diet) was recently reported (ISABEL et al., 1999). Results of these authors are more in agreement with our results when a lower reflectance at 630 nm and significant ($P<0.05$) difference between reflectance at 580 and 630 nm were received from *psoas major* muscle after refrigerated storage for 7

days of pigs supplemented with 200 mg vitamine E/kg^{-1} feed for 60 days before slaughtering. Different muscles (*psoas major* with overall lower reflectance values when compare to *longissimus dorsi* muscle in our experiment, Table 2) could exhibit different rates of oxymyoglobin oxidation (i.e. discoloration) when displayed under different condition and vitamin E treatment increased oxymyoglobin stability in *range psoas major*, *gluteus medius* and *longissimus dorsi* beef muscles stored at 4° C (CHAN et al., 1996). Colour stability and lipid oxidative processes in fresh meat are known to be closely related and low muscle vitamin E levels resulted in low 'a' values and concurrent high rates of lipid oxidation (JENSEN et al., 1998). Our findings of positive vitamin E effects on colour differences in psoas major muscle (refrigerated storage for 7 days) are supported by significant ($P<0.05$) lower TBARS values (Table 2) in pigs supplemented with vitamin E and organic Se as well. We did not receive significant differences ($P>0.05$) on TBARS values of *longissimus dorsi* muscle (refrigerated storage for 7 days) of pigs supplemented with vitamin E and organic Se (Table 2) but the rate of iron-induced lipid oxidation of LD was strongly influenced by dietary vitamin E. However, as shown in the Figure, the oxidative changes on LD were

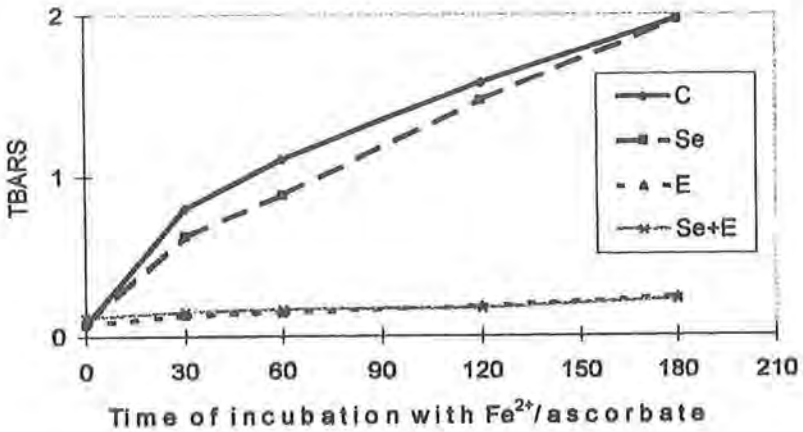

Fig.: Time course of Fe^{2+}/ascorbate induced lipid peroxidation of *longissimus dorsi* muscle post mortem of control and supplemented (vitamin E, organic selenium and vitamin E + organic selenium) pigs. All differences between supplemented with vitamin E groups and groups not supplemented with vitamin E are significant ($P < 0.01$) (Zeitlicher Verlauf der Lipidperoxidation (TBARS) in Muskelhomogenaten (LD) nach Inkubation mit Fe^{2+}/Ascorbat)

not influenced by dietary addition of organic Se. The effect of vitamin E was statistically significant ($P<0.05$) in muscle (*longissimus dorsi*) homogenate prepared from samples post mortem. It was shown previously that the oxidative changes were large in LD of pigs fed diets not supplemented with dl-α-tocopheryl acetate compared with LD of pigs supplemented with dl-α-tocopheryl acetate at incubation for 120 and 160 min (LAURIDSEN et al., 1999). Supplementation of organic Se does not affect the oxidative stability of muscle tissue (*longissimus dorsi*) when the rate of iron-induced lipid oxidation of LD was examined and the time course of stimulation with Fe^{2+} does not differ from control pigs (Fig.). GRADY et al. (1998) were not able to

find differences on muscle Se-GSH-Px activity between control and the dietary Se supplemented pigs. Elevating dietary Se (0.3 mg/kg^{-1} diet), while adhering to regulations relating to dietary Se does not affect the oxidative stability of beef.
In the literature there are data about more efficiency on clinical signs of cows when supplemented with both vitamin E and Se but their action was not additive and it is supposed glutathione peroxidase activity (related to Se administration) may spare the requirement for vitamin E in the membranes (SMITH et al., 1997). From our results follows that dietary Se has limited potential for enhancing the quality of pork carcass (*psoas major*) and accentuating the effect of vitamin E on oxidative stability of *longissimus dorsi* muscle was not found.

Conclusion

The results showed that administration of organic bound Se (0.3 mg/kg feed for 60 days) could in some extend positively influence colour and oxidative stability but as expected supplementation with vitamin E (200 mg/kg^{-1} feed for 60 days) to finishing pigs is more effective in improving the antioxidative defence system in carcasses of pigs. We concluded that high dietary level of vitamin E to pigs increases the color stability and stability of fat in carcasses of pigs.

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5. vollständig neubearbeitete Auflage, 915 Seiten, 100 Tabellen, 332 Abbildungen, Parey Buchverlag im Blackwell Wissenschaftsverlag Berlin, 2000, ISBN: 3-8263-3178-8
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Dieses von Josef Bloch und Rudolf Supperer begründete Standardwerk liegt in seiner 5. vollständig neubearbeiteten Auflage vor. Sowohl die Erkenntnisfortschritte in der Parasitologie z.B. im Bereich der immunologischen und molekularen Diagnostik, der Epidemiologie, der Therapie als auch die fehlende Verfügbarkeit der 1991 erschienenen 4. Auflage dieses Standardwerkes im Verlag, machten diese Neuauflage dringend erforderlich. Das kompetente Autorenteam aus Österreich, der Schweiz und Deutschland nutzte beide Gründe für eine vollständige Neubearbeitung. Unter Beibehaltung der bewährten Grundgliederung, wurde der Text des Buches ohne Schmälerung des Informationswertes, unter dem Aspekt von Fortschritten in der praktischen Diagnostik und der Relevanz für die Bekämpfung von Parasitosen, gestrafft. Neben der Einarbeitung neuer Erkenntnisse wurden Schemazeichnungen eingefügt, eine umfangreiche nach Tierarten geordnete Antiparasitenliste zusammengefaßt und eine notwendigerweise gestraffte, sehr sachkundige Literaturauswahl getroffen.
Das Buch enthält im ersten allgemeinen Teil die Abschnitte Symbiose und Parasitismus, die Erreger von Parasitosen mit Systematik sowie Taxonomie und allgemeine Merkmale (beides neue Abschnitte), Parasit-Wirt-Beziehungen, Untersuchungsmethoden und Parasitenstadien als umwelthygienisches Problem. Der spezielle Teil gliedert sich in Parasitosen der Wiederkäuer (Rind, Schaf, Ziege), Einhufer (Pferd, Esel), Schwein, Hund und Katze, Kaninchen, Nutzgeflügel (Huhn, Truthuhn, Gans, Ente, Taube),Wild (Wildwiederkäuer, Wildschwein, Hase, Fasan und Rebhuhn), Süßwassernutzfische sowie Bienen. Innerhalb der Tierarten sind die Parasitosen wo notwendig nach Protozoeninfektion, Helminthosen, Pentastomidenbefall und Arthropodenbefall geordnet. Bei jeder Parasitose werden in erforderlichem Umfang meist Erreger, Vorkommen und Verbreitung, Entwicklung und Epidemiologie, Pathogenese, Pathologie, Klinik, Diagnose, Therapie, Bekämpfung und wo erforderlich, auch die Bedeutung für den Menschen, beschrieben. Den Erregergruppen ist ein aktualisiertes Verzeichnis neuester Literatur beigefügt. Die sich auf das wesentliche und notwendige beschränkende, klare Textfassung, zahlreiche Abbildungen, schematische Übersichten und Tabellen ermöglichen eine große Information. Ein Anhang enthält die Zusammenstellung der Antiparasitika für die einzelnen Tierarten, einschließlich der Dokumentation ihres Zulassungsstatus in Österreich, Schweiz und Deutschland sowie Begriffsdefinitionen, Abkürzungs- und Sachwortverzeichnis.
Als Standardwerk der Parasitologie ist es unentbehrlich für Untersuchungsämter, Gesundheitsdienste, wissenschaftliche Einrichtungen der Veterinärmedizin, Tierzucht und -haltung und weitere biologische Fachrichtungen. Es vermittelt einerseits Studierenden in konzentrierter Form morphologische und biologische Einzelheiten der Erreger und ihrer Bekämpfungsmöglichkeiten und ebenfalls grundlegende Angaben über Systematik, Pathogenese, Wirt-Parasit-Verhältnis und parasitologische Nachweismethoden. Andererseits ist es als Arbeitsmittel und spezielles Nachschlagewerk für den praktizierenden Tierarzt, der sich für seine tägliche Arbeit über Epizootologie, Klinik, Diagnose, Therapie und Prophylaxe der verschiedenen Parasiten orientieren will, unverzichtbar. Dieses Buch bedarf eigentlich keiner besonderen Empfehlung.

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