



Gut Feelings: How Probiotics May Improve Animal Welfare

“**Probiotic**” has recently become a buzzword. It is heard in relation to yogurt and supplements, and seen on television commercials and billboards. The public is told over and over that probiotics are good for the body. However, how many people know that probiotics are found naturally in the bodies of animals and humans all over the world? How many know that probiotics can be used to improve the lives of livestock and lab animals? In this Extension bulletin, we will discuss probiotics that exist in the gut of mammals, chemicals produced by these probiotics, implications for animal welfare, and how to select and store probiotics.

Elizabeth Petrosus, Animal Sciences, Purdue University

Susan Eicher, USDA-ARS Livestock Behavior Research Unit

Marisa Erasmus, Animal Sciences, Purdue University

Introduction

On farms and in labs, caretakers take precautions to consider the welfare of their animals. According to Broom (1986), animal welfare is “the state of an animal regarding its attempts to cope with its environment.” Fraser et al. (1997) suggest that three main ideas contribute to animal welfare: “biological functioning, subjective experience, and natural living.” Biological functioning is the overall physical health of the animal. Subjective experiences, or affective states, account for the feelings or emotions that animals may experience. Natural living refers to the animal’s ability to express its instinctual and natural behaviors. In captive and production-intensive environments, animals may be at risk of decreased welfare.

In some cases, supplementation of probiotics may help improve welfare by reducing negative affective states. However, before supplementing animals with probiotics, it is important to understand how probiotics work.

Microorganisms exist in many locations in and on the bodies of animals, including the skin, respiratory system, and gastrointestinal tract (gut). In each location, pathogenic, commensal, and probiotic microorganisms exist. Commensal microorganisms are “microbes that induce either no damage or clinically inapparent damage after primary infection” (Casadevall and Pirofski, 2000). Pathogens can cause infection or disease. In contrast, probiotics are defined as microorganisms that are beneficial to the body when provided in sufficient amounts (FAO/WHO, 2001). Direct Fed Microbials (DFM) is a term used in the livestock industry and may include dead as well as live microorganisms.

Which probiotic species live in the gut of mammals?

The species of microorganisms that live in an animal's gut differ from individual to individual due to differences in diet, environment, and habits. However, patterns have accrued over time. Gut microbes have coevolved with mammals, and by examining the microorganisms that live in the gut of 60 mammalian species, it has been concluded that individuals of the same species have more similar gut compositions than members of different species (Ley et al., 2008). Because of their more complex diets, herbivores contained the highest diversity of microorganisms, with carnivores having the least diversity and omnivores being intermediate (Ley et al., 2008). Therefore, it is critical to remember that as different species evolved, their gut microorganisms evolved as well, with different species likely having dissimilar microbiomes.

In addition to commensal and pathogenic bacteria, there are probiotic species that naturally reside in the gut of mammals. Table 1 outlines the known probiotic microorganisms found in common livestock and lab animal species.

Notice that some of the same probiotic species are commonly found in the guts of different mammalian species. For example, *Bacillus* spp., *Bacteroides* spp., *Clostridium* spp., *Enterococcus* spp., *Eubacterium* spp., *Faecalibacterium* spp., *Lactobacillus* spp., and *Prevotella* spp. are seen in at least three of the five animal species listed. These microbes may be ideal candidates for probiotic research due to the applicability across multiple species.

Neurochemicals produced by probiotics

In the gut, probiotics can produce varied effects on the body, ranging from pathogen resistance and prevention of diarrhea to reducing anxiety-like and depression-like behaviors. Behavioral effects are facilitated through the gut-brain axis (GBA). The GBA is a connection between the gut and the brain through the vagus nerve (Bercik et al., 2011; Bravo et al., 2011). Through this nerve, the gut and the brain communicate. Some probiotic species produce chemicals that act on the brain, or neurochemicals (Lyte, 2011). Neurochemicals are molecules that are involved in neural activity and regulate brain functions. When these neurochemicals are produced in the gut, it can initialize changes to occur in the brain, resulting in alterations in behavior.

Neurochemicals can also be produced by probiotics, or beneficial microorganisms, in the gut and, via the gut-brain axis, influence a wide range of brain processes, including memory, anxiety, arousal, and stress. Gamma-aminobutyric acid (GABA) is a neurotransmitter and amino acid found in nearly all eukaryotic (animal) and prokaryotic (microbial) organisms (Bown and Shelp, 1997). In animals, GABA inhibits neuron transmission. This inhibitory action reduces symptoms of neural disorders that are associated with hyperactivity of the neurons, such as anxiety. GABA is

Table 1. Probiotic species found in the gut of mammals

Species of Animal	Probiotics found	References
Swine	<i>Bacillus</i> spp., <i>Clostridium</i> spp., <i>Eubacterium</i> spp., <i>Lactobacillus</i> spp., <i>Prevotella</i> spp., <i>Streptococcus</i> spp.	Leser et al., 2002 Lamendella et al., 2011
Cattle	<i>Bacteroides</i> spp., <i>Clostridium</i> spp., <i>Enterococcus</i> spp. <i>Faecalibacterium</i> spp., <i>Lactobacillus</i> spp., <i>Prevotella</i> spp.	Dowd et al., 2008 Malmuthuge et al., 2014
Chickens	<i>Bacillus</i> spp., <i>Bacteroides</i> spp., <i>Clostridium</i> spp., <i>Enterococcus faecium</i> , <i>Escherichia coli</i> , <i>Eubacterium</i> spp., <i>Faecalibacterium prausnitzii</i> , <i>Lactobacillus</i> spp., <i>Staphylococcus</i> spp.	Bjerrum et al., 2006 Gong et al., 2007
Macaques	<i>Bacteroides</i> spp., <i>Bifidobacterium</i> spp., <i>Lactobacillus</i> spp., <i>Prevotella</i> spp.	McKenna et al., 2008 O'Sullivan et al., 2013
Humans	<i>Acinetobacter</i> spp., <i>Bacillus</i> spp., <i>Bacteroides</i> spp., <i>Bifidobacterium</i> spp., <i>Clostridium</i> spp., <i>Enterococcus</i> spp., <i>Escherichia coli</i> , <i>Eubacterium</i> spp., <i>Faecalibacterium prausnitzii</i> , <i>Lactobacillus</i> spp., <i>Oxalobacter formigenes</i> , <i>Parabacteroides</i> spp., <i>Pediococcus acidilactici</i> , <i>Prevotella</i> spp., <i>Streptococcus</i> spp.	Methé et al., 2012

desirable and is produced by *Lactobacillus* spp., and *Bifidobacterium* spp.

Norepinephrine (NE) is a neurotransmitter that regulates arousal and the stress response (Goddard et al., 2010). Administration of NE can result in increased blood pressure, respiration, heart rate, and anxiety (Bremner et al., 1996; Rogeness et al., 1990). Therefore, high levels of NE are undesirable. Norepinephrine can be produced by *Escherichia* spp., *Bacillus* spp., and *Saccharomyces* spp.

Serotonin is a neurotransmitter that influences mood functions ranging from aggression to anxiety (Holmes et al., 2003; Näslund et al., 2016; Näslund et al., 2015; Seo et al., 2008). Low levels of serotonin are associated with depression or depression-like behaviors (Sullivan et al., 2006; Owens and Nemeroff, 1994). In humans these conditions are treated with selective serotonin reuptake inhibitors (SSRI), which leave more free serotonin available in the body. SSRIs are associated with anti-depressive and anxiolytic, or anxiety reducing, behaviors (Papakostas et al., 2008). Therefore, maintaining adequate levels of serotonin is important for treating depression and anxiety. Serotonin can be produced by *Candida* spp., *Streptococcus* spp., *Escherichia* spp., and *Enterococcus* spp.

Similar to serotonin, dopamine is a neurotransmitter that regulates many processes, including reward, motivation, and addiction. Neural disorders, such as depression, anxiety, schizophrenia, bipolar disorder, and Parkinson's disease, have been linked to dopamine dysregulation (Berk et al., 2007; Weintraub et al., 2005). Thus, dopamine is desirable and is produced by *Bacillus* spp., and *Serratia* spp.

Acetylcholine is a neurotransmitter that plays a critical role in learning and the formation of new memories. When acetylcholine receptors are blocked, old memories can still be retrieved, but new memories cannot be formed (Atri et al., 2004). When drugs that open acetylcholine receptors are administered, new memory formation is enhanced (Levin et al., 2006). Acetylcholine is a desirable neurochemical and can be produced by *Lactobacillus* spp.

Implications for animal welfare

Animals in captive environments may be at risk of negative mental states, often showing depression-like and anxiety-like behaviors (Boissy and Lee, 2014; Douglas et al., 2012; Bateson and Matheson, 2007; Fraser, 1988). Negative mental states may be perpetuated by a lack of ability to perform natural behaviors, lack of stimulation, excess noise, odors, artificial light, and other environmental factors that are beyond the



control of the animal (Bateson and Matheson, 2007; Morgan and Tromborg, 2007; Mench, 1998; Friend, 1989). Also, husbandry practices such as weaning, identification tagging, tail docking, and teeth clipping can cause stress and negative mental states (Marchant-Forde et al., 2009; Grandin, 1997; Dantzer and Mormede, 1983).

Changing the captive environment is one way to improve the animal's welfare. However, factors such as space and financial limitations may prevent caretakers from making environmental changes, and husbandry practices are necessary and potentially unavoidable. In such cases, probiotics may be able to reduce negative mental states and improve animal welfare.

When neurochemicals are released by probiotics in the gut, they communicate with the brain through the vagus nerve. The presence of these neurochemicals induces changes in the brain and behavior. For example, high levels of GABA, serotonin, and dopamine, and low levels of norepinephrine have anxiety-reducing effects. Finding a probiotic mixture that produces these levels of neurochemicals can provide an anxiety-reducing effect for the animals, which may improve animal welfare. Providing the right probiotic mixtures may improve the animal's welfare by reducing negative affective states.

Probiotic selection and storing

If you've decided that probiotic supplementation is appropriate for improving the welfare of your animals, here are some points to consider.

When selecting a probiotic supplement or mixture for use on your animals, do your research. Probiotic species and

strains within species have different effects on the body; look for the species and strain that suit your needs. Some probiotics will not survive in all species of animals. Table 1 can be used as a reference for which probiotic species already exist for different animal species.

For most supplements to work, the probiotic microorganisms will need to be alive. Look on the supplement's nutritional label for the company's guarantee that the probiotic will be alive until the date of expiration (Reid et al., 2003). In order to have effects on the animal, there needs to be enough probiotic microorganisms in each capsule or dose. Look on the nutritional label and confirm that the amount included in each capsule or dose is within the range of 1 billion to 10 billion colony-forming units (CFUs) (Reid et al., 2001; Reid et al., 2003).

It is recommended to keep probiotics in a cool, dry place. Look on the label for specific storing instructions; some probiotics need to be kept refrigerated. Always follow the instructions and correct dosage on the packaging of your individual probiotic supplement or mixture. If unsure of which probiotic to supplement your animals, consult with your nutritionist or veterinarian. It is recommended to observe the behavior of your animals after probiotic supplementation. If adverse behaviors are observed, contact your veterinarian immediately.

Glossary of terms and abbreviations

- **Anxiolytic:** anxiety reducing
- **Carnivore:** animal that only eats meat
- **Eukaryote:** cell that contains a nucleus and membranous organelles; refers to animals and plants
- **Gastrointestinal tract:** the gut; consists of the stomach, small intestines, large intestines, and rectum
- **Herbivore:** animal that eats only plants
- **Microbes:** microorganisms
- **Microbiome:** all microorganisms in a particular location
- **Omnivore:** animal that eats both plants and animals
- **Prokaryote:** cell that lacks a nucleus and membranous organelles; refers to bacteria
- **spp.:** abbreviation that means species; used in microbiology to describe a group of microorganisms; example: *Lactobacillus* spp. = *Lactobacillus* species, species of microorganisms in the genus *Lactobacillus*

References

- Atri, A., S. Sherman, K.A. Norman, B. A. Kirchoff, M. M. Nicolas, M.D. Greicius, S.C. Cramer, H.C. Breiter, M.E. Hasselmo, and C.E. Stern. 2004. Blockade of Central Cholinergic Receptors Impairs New Learning and Increases Proactive Interference in a Word Paired-Associate Memory Task. *Behav. Neurosci.* 118(1): 223-236.
- Bateson, M., and S.M. Matheson. 2007. Performance on a categorisation task suggests that removal of environmental enrichment induces 'pessimism' in captive European starlings (*Sturnus vulgaris*). *Anim. Welf.* 16(5): 33-36.
- Bercik, P., A.J. Park, D. Sinclair, A. Khoshdel, J. Lu, X. Huang, Y. Deng, P.A. Blennerhassett, M. Fahnestock, D. Moine, B. Berger, J.D. Huizinga, W. Kunze, P.G. McLean, G.E. Bergonzelli, S.M. Collins, and E.F. Verdu. 2011. The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut-brain communication. *J Neurogastroenterol. Motil.* 23:1132-e544.
- Berk, M., S. Dodd, M. Kauer-Sant'Anna, G.S. Malhi, M. Bourin, F. Kapczinski, and T. Norman. 2007. Dopamine dysregulation syndrome: implications for a dopamine hypothesis of bipolar disorder. *Acta Psychiatrica Scandinavica.* 116(suppl. 464): 41-49.
- Bjerrum, L., R.M. Engberg, T.D. Leser, B.B. Jensen, K. Finster, and K. Pedersen. 2006. Microbial Community Composition of the Ileum and Cecum of Broiler Chickens as Revealed by Molecular and Culture-Based Techniques. *Poult. Sci.* 85: 1151-1164.
- Boissy, A., and C. Lee. 2014. How assessing relationships between emotions and cognition can improve farm animal welfare. *Rev. Sci. Tech. Off. Int. Epiz.* 33(1): 103-110.
- Bown, A.W., and B.J. Shelp. 1997. The Metabolism and Functions of γ -Aminobutyric Acid. *Plant Physiol.* 115: 1-5.
- Bravo, J., P. Forsythe, M. Chew, E. Escaravage, H. Savignac, T. Dinan, J. Bienenstock, and J. Cryan. 2011. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in the mouse via the vagus nerve. *PNAS.* 108:16050-16055.
- Bremner, J.D., J.H. Krystal, S.M. Southwick, and D.S. Charney. Noradrenergic Mechanisms in Stress and Anxiety: II Clinical Studies. *Synapse.* 23: 39-51.
- Broom, D.M. 1986. Indicators of Poor Welfare. *Brit. Vet. J.* 142(6): 524-526.
- Dantzer, R., and P. Mormède. 1983. Stress in Farm Animals: A Need of Reevaluation. *J. Anim. Sci.* 57: 6-18.
- Douglas, C., M. Bateson, C. Walsh, A. Bédue, and S.A. Edwards. 2012. Environmental enrichment induces optimistic cognitive biases in pigs. *Appl. Anim. Behav. Sci.* 139(1-2): 65-73.
- Dowd, S.E., T.R. Callaway, R.D. Wolcott, Y. Sun, T. McKeenan, R.G. Hagevoort, and T.S. Edrington. 2008. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *BMC Microbiol.* 8: 125.
- Fraser, A.F. 1988. Animal suffering: the appraisal and control of depression and distress in livestock. *Appl. Anim. Behav. Sci.* 20: 127-133.
- Fraser, D., D. Weary, E. Pajor, and B. Milligan. 1997. A Scientific Conception of Animal Welfare That Reflects Ethical Concerns. *Anim. Welf.* 6:187-205.
- Friend, T.H. 1989. Recognizing behavioral needs. *Appl. Anim. Behav. Sci.*, 22: 151-158.
- Goddard, A.W., S.G. Ball, J. Martinez, M. J. Robinson, C.R. Yang, J.M. Russel, and A. Shekhar. 2010. Current perspectives of the roles of the central norepinephrine system in anxiety and depression. *Depress. Anxiety.* 27: 339-350.
- Gong, J., W. Si, R.J. Forster, R. Huang, H. Yu, Y. Yin, C. Yang, and Y. Han. 2007. 16S rRNA gene-based analysis of mucosa-associated bacterial community and phylogeny in the chicken gastrointestinal tracts: from crops to ceca. *FEMS Microbiol. Ecology.* 59: 147-157.
- Grandin, T. 1997. Assessment of Stress During Handling and Transport. *J. Anim. Sci.* 75: 249-257.
- Holmes, A., D.L. Murphy, and J.N. Crawley. 2003. Abnormal behavioral phenotypes of serotonin transporter knockout mice: parallels with human anxiety and depression. *Biol. Psychiatry.* 54(10): 953-959.
- Lamendella, R., J.W. Santo Domingo, S. Ghosh, J. Martinson, and D.B. Oerther. 2011. Comparative fecal metagenomics unveils unique functional capacity of the swine gut. *BMC Microbiol.* 11:103.



Leser, T.D., J.Z. Amenuvor, T.K. Jensen, R.H. Lindecrone, M. Boye, and K. Möller. 2002. Culture-Independent Analysis of Gut Bacteria: the Pig Gastrointestinal Tract Microbiota Revisited. *Appl. Environ Microbiol.* 68(2): 673-690.

Levin, E.D., F.J. McClernon, and A.H. Rezvani. 2006. Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. *Psychopharmacol.* 184(3): 523-539.

Lyte, M. 2011. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the design and use of probiotics. *Bioessays.* 33: 574-581.

Malmuthuge, N., P.J. Griebel, and L.L. Guan. 2014. Taxonomic Identification of Commensal Bacteria Associated with the Mucosa and Digesta throughout the Gastrointestinal Tracts of Preweaned Calves. *Appl. Environ. Microbiol.* 80(6): 2021-2028.

Marchant-Forde, J.N., D.C. Lay Jr., K.A. McMunn, H.W. Cheng, E.A. Pajor, and R.M. Marchant-Forde. 2009. Postnatal piglet husbandry practices and well-being: The effects of alternative techniques delivered separately. *J. Anim. Sci.* 87:1479-1492.

McKenna, P., C. Hoffmann, N. Minkah, P.P. Aye, A. Lackner, Z. Liu, C. A. Lozupone, M. Hamady, R. Knight, and F.D. Bushman. 2008. The Macaque Gut Microbiome in Health, Lentiviral Infection, and Chronic Enterocolitis. *PLOS Pathogens.* 4(2): 1-12.

Mench, J. 1998. Why it is important to understand animal behavior. *ILAR J.* 39(1): 20-26.

Méthé, B.A., K.E. Nelson, M. Pop, H.H. Creasy, M.G. Giglio, C. Huttenhower, and O. White. (2012). A framework for human microbiome research. *Nature*, 486(7402), 215-221. <http://doi.org/10.1038/nature11209>.

Morgan, K.N., and Tromborg, C.T. 2007. Sources of stress in captivity. *Appl. Anim. Behav. Sci.* 102(3-4): 262-302.

Näslund, J., E. Studer, R. Pettersson, M. Hagsäter, S. Nilsson, H. Nissbrandt, and E. Eriksson. 2015. Differences in Anxiety-Like Behavior within a Batch of Wistar Rats Are Associated with Differences in Serotonergic Transmission, Enhanced by Acute SRI Administration, and Abolished by Serotonin Depletion. *Int. J. Neuropsychopharmacol.* DOI: <http://dx.doi.org/10.1093/ijnp/pyv018>. p. 1-9.

Näslund, J., E. Studer, E. Johansson, and E. Eriksson. 2016. Effects of gonadectomy and serotonin depletion on inter-individual differences in anxiety-like behaviour in male Wistar rats. *Behav. Brain Res.* 308: 160-165.

O'Sullivan, A., X. He, E.M.S. McNiven, N.W. Haggarty, B. Lönnerdal, and C.M. Slupsky. 2013. Early Diet Impacts Infant Rhesus Gut Microbiome, Immunity, and Metabolism. *Am. Chem. Soc.* 12: 2833-2845.

Owens, M.J., and C.B. Nemeroff. 1994. Role of Serotonin in the Pathophysiology of Depression: Focus on the Serotonin Transporter. *Clin. Chem.* 40(2): 288-295.

Papakostas, G.I., S.M. Stahl, A. Krishen, C.A. Seifert, V.L. Tucker, E.P. Goodale, and

M. Fava. 2008. Efficacy of Bupropion and the Selective Serotonin Reuptake Inhibitors in the Treatment of Major Depressive Disorder With High Levels of Anxiety (Anxious Depression): A Pooled Analysis of 10 Studies. *J. Clin. Psychiatry.* 69(8): 1287-1292.

Reid, G., D. Beuerman, C. Heinemann, and A.W. Bruce. 2001. Probiotic Lactobacillus dose required to restore and maintain a normal vaginal flora. *FEMS Immunol. Med. Microbiol.* 32(1):37-41.

Reid, G., M.E. Sanders, H.R. Gaskins, G.R. Gibson, A. Mercenier, R. Rastall, M. Roberfroid, I. Rowland, C. Cherbut, and T.R. Klaenhammer. 2003. New Scientific Paradigms for Probiotics and Prebiotics. *Journal of Clinical Gastroenterology.* 37(2): 105-118.

Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. 2001. Health and Nutrition Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Cordoba, Argentina.

Rogeness, G.A., C. Cepeda, C.A. Macedo, C. Fisher, and W.R. Harris. 1990. Differences in heart rate and blood pressure in children with conduct disorder, major depression, and separation anxiety. *Psychiatry Res.* 33(2): 199-206.

Seo, D., C.J. Patrick, and P.J. Kennealy. Role of serotonin and dopamine system interactions in the neurobiology of impulsive aggression and its comorbidity with other clinical disorders. *Aggress. Violent Behav.* 13(5): 383-395.

Sullivan, G.M., J.J. Mann, M.A. Oquendo, E.S. Lob, T.B. Cooper, and J.M. Gorman. 2006. Low Cerebrospinal Fluid Transthyretin Levels in Depression: Correlations with Suicidal Ideation and Low Serotonin Function. *Biol. Psychiatry.* 60(5): 500-506.

Weintraub, D., A.B. Newberg, M.S. Cary, A.D. Siderowf, P.J. Moberg, G. Kleiner-Fisman, J.E. Duda, M.B. Stern, D. Mozley, and I.R. Katz. 2005. Striatal Dopamine Transporter Imaging Correlates with Anxiety and Depression Symptoms in Parkinson's Disease. *J. Nucl. Med.* 46(2): 227-232.