Feeding the world:
Arkansas lab uses IPA to improve feed efficiency in chickens

At the University of Arkansas, Walter Bottje has been investigating how mitochondrial function affects muscle growth in chickens to help alleviate a looming food shortage. For this important work, Bottje's lab used Ingenuity® Pathway Analysis™ to understand the biological relevance of gene expression in feed efficiency.

The world faces a growing shortage of its food supply, and scientist Walter Bottje is working on the front lines to prevent a global crisis. By 2050, it's predicted that the world population will require twice as much food as we produced in 2010. Even worse, due to limitations of arable land, only about 10 percent of that increase is expected to come from growing more crops. The other 90 percent will have to come from making food production more efficient.

And that's where Bottje, a faculty member in the Poultry Science department at the University of Arkansas, has set his sights. With his longtime interest in mitochondria, he and collaborators including Byung Whi Kong are tackling the question of how to use biological insights to improve feed efficiency in chickens. "One of the key genetic selection programs that they have in commercial breeding operations is trying to improve feed efficiency, which is how much feed does it take to get a pound of animal — more specifically, a pound of meat," Bottje says.

In a recent study, his team analyzed chickens from a commercial breeding program, looking for differentially expressed genes and proteins that could explain why some birds grow significantly larger than others while eating the same amount of feed. Bottje used Ingenuity Pathway Analysis (IPA) and found certain proteins and pathways upregulated in the high-efficiency chickens but not in the low-efficiency group. From Bottje's perspective, taking advantage of this information can't happen quickly enough.

"It takes five years for the effects of any new genetic selection to reach the commercial broiler," he says, underscoring the need to quickly find genomic or proteomic insights to meet the 2050 food challenge.

Exploring mitochondria

Bottje's lab focuses primarily on mitochondria, which have been associated with feed efficiency in the past. The team has been studying broilers, or chickens raised for meat, and pioneered the concept of studying feed efficiency differences in a single genetic inbred line of birds. That helps take other factors affecting mitochondria — such as diet and fat or lean body types — out of the equation to keep the emphasis on genes and proteins of interest. Since then, the approach has been applied to several other different livestock animals.

The University of Arkansas is located near a large commercial breeding company, giving

Bottje access to chickens worth analyzing. For a recent study, his lab and collaborators in Australia evaluated the top four and bottom four performers in the company's feed efficiency program — that is, chickens that gained the most or least on the same amount of feed — to find differences at the cellular level that could explain this phenotypic outcome. "What's making these animals more efficient?" Bottje wondered, and targeted his study at muscle tissue. "Muscle is important because it contributes about 40 percent of basal metabolic rate in animals, so it can have a lot of impact on overall efficiency." The goal, he notes, is to identify key drivers in feed efficiency to help breeding companies improve their genetic selection process.

For this project, Bottje and his team conducted two global gene expression studies, first with microarrays and later with RNA-seq. They also incorporated a shotgun proteomics data set generated from the same sets of tissue. The combination of data sets was particularly important for highlighting areas of overlap and divergence. "It's really beneficial to us to be able to overlay the data sets with the IPA program and look at proteogenomics associated with this particular trait," Bottje says.

Analyses showed clear differences between mitochondrial function in the high- and low-efficiency chickens, and the proteomics data corroborated this. "It's a validation of what we've been seeing for quite a long time in our research," Bottje says. "In the proteomics data set we saw that four of the five complexes had higher expression in the high feed efficiency, where they were predicted to be activated [by IPA]."

In the study, 152 of 1,800 proteins were differentially expressed between the sets of birds. Across all proteins, the collective expression of more than 200 mitochondrial proteins was associated with higher expression in the more efficient chickens. When IPA crunched the data, the mitochondrial dysfunction pathway had the most differentially expressed genes between the groups, with significant up-regulation in the highly efficient set of chickens. Using IPA's Upstream Regulator Analysis, Bottje's team learned that insulin receptor, nuclear factor erythroid 2-like 2, progesterone, and other genes would be activated in the high-efficiency birds while serum response factor and other genes would be inhibited.

The use of IPA and another analytical tool developed by collaborators Toni Reverter and Nick Hudson in Australia led Bottje to the idea of mitochondrial hormone receptors, which allow for direct signaling between mitochondria and hormone receptors without using the nucleus as an intermediary. "When I started looking around the avian literature, there was nothing on mitochondrial hormone receptors," he says. "Now it's very apparent that you can have direct signals. We've started to characterize these and find out if they're functional."

Evidence suggests there could be other surprises as well. "There may be a bunch of other non-traditional hormones that could be affecting mitochondrial function and I think the only way we got there was through the IPA program," Bottje says. "There's a whole bunch of communication going on in the cell

that researchers are just not aware of, and this could be highly relevant to feed efficiency."

Now the team is moving ahead with a hypothesis-generating approach, starting with predictions of activation from tools such as IPA to form a hypothesis and then analysis of downstream targets or in vitro studies to support or refute it.

Unexpected findings

Bottje is an experienced IPA user who says the software is useful for anyone conducting global gene expression studies. "It's been daunting to try to approach these studies on a holistic basis. You get these phenomenal data sets that have all this information in them, but then it's how can you pull information out of them," he says. "IPA has really helped in terms of laying out some of these pathways."

The application also saves him quite a bit of time. Bottje recalls experiments prior to IPA where each new gene or protein he'd uncover would lead to days of research to figure out how it might be relevant. "IPA has really helped in organizing thoughts. A couple of clicks, and I'm into the literature," he adds.

Other programs may highlight bundles of genes but fail to shed light on physiology or potential relevance to Bottje's area of interest. "It would be very difficult to do this with other programs that are out there," he says. "With IPA, you may end up finding out things about your particular subject area that you wouldn't have gotten to otherwise."

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